CEREAL CHEMISTRY

CONTENTS

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Why ENRICH Cereal foods ?

The purpose of enrichment is "to correct major faults in many American diets, namely, inadequacy in thiamine, riboflavin, niacin and iron." (*BREAD and FLOUR ENRICHMENT, 1946-47, Prepared by the Committee on Cereals, Food and Nutrition Board, National Research Council.*)

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THE ENVIRONMENTAL AND AGRONOMICAL FACTORS INFLUENCING THE THIAMINE, RIBOFLAVIN, NIACIN, AND PANTOTHENIC ACID CONTENT OF WHEAT, CORN, AND OATS [1]

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ABSTRACT

Data compiled on the thiamine, riboflavin, niacin, and pantothenic acid contents of wheat, corn, and oats grown on three types of soil fertility experiments during several seasons were subjected to statistical analysis. Interpretation as to the effect of season (year in which crop was grown), lime, and various types of fertilizers were made on the basis of trends or statistically significant differences.

In the five-year rotation fertility experiment, season affected the thiamine and riboflavin content of wheat, corn, and oats, the niacin content of corn and oats, and the pantothenic acid content of corn.

Liming the soil in the five-year rotation experiment increased the thiamine content of wheat, corn, and oats, and the niacin and pantothenic acid content of wheat. Liming the soil also decreased the hull and niacin content of oats in 1944 (dry year). This decrease was less significant where a complete fertilizer (NPK) was used in comparison with single fertilizers (N or P or K).

The effect of fertilizers (other than lime) on the vitamin content of wheat was selective. Phosphorus alone appears to have increased the thiamine content, while nitrates increased the niacin and nitrates and potash in combination increased the pantothenic acid content of wheat. Potash alone appears to have decreased the thiamine content of wheat.

Nitrates alone significantly increased the thiamine content of oats. No other fertilizer effect, other than lime, was apparent.

The thiamine content of corn appears to have been decreased by potash as a single fertilizer. These results were apparent for two years. The thiamine content of corn grown during a dry season (1944) was less than the thiamine content of corn grown during a normal season.

Season affected the thiamine content of wheat and oats in the continuous culture experiment, but, unlike that found in the five-year rotation experiment, the thiamine content of corn was not affected. Wheat grown in a

normal year (1948) had a higher riboflavin content than wheat grown in a wet year (1947). Reverse results were found in oats.

Liming the soil did not significantly increase the thiamine content of of oats nor increase the thiamine content of corn. These results are the reverse of those found in the five-year rotation experiment.

The application of variable amounts of a 2-12-6 fertilizer produced no significant effect on the vitamin B complex content of wheat. Wheat grown on the "rate of fertilization experiment," of recent origin, had a higher average thiamine, riboflavin, niacin, and pantothenic acid content than wheat grown in the five-year rotation experiment and a greater niacin content than wheat grown in the continuous culture experiment.

Plants and their fruits supply almost the entire feed of animals and a large part of the food for man. Any information concerning factors which influence the value of plants and their fruits (grains) as a source of nutrients is of great nutritional and national importance.

It has been established that the application of certain fertilizers to the soil affects the composition of wheat, Ames (1, 2); Hunt (7). The question arises as to whether there is a correlation between the members of the vitamin B-complex and the changes in the other chemical components of the grain. Some investigators have observed that the vitamin B content is correlated with the phosphorus content of the grain, Voegtlin and Meyers (17), and that wheat grown on a soil fertilized with phosphate had a higher vitamin B content than wheat grown without phosphate fertilizer, Hunt (7). Rowlands and Wilkinson (12) have shown that soils treated with barnyard manure produced seeds of a higher vitamin B content than those produced with artificial commercial fertilizers. Since the above observations were made, vitamin B has been found to be a complex and nothing is known as to what factor or factors of the complex may be influenced by such soil treatment.

Schultz, Atkin, and Frey (14) have shown that there are significant differences in the thiamine content of different varieties of the same cereal and that there are indications that regional differences may affect the thiamine content of a single variety. Downs and Cathcart (5) found that hard wheats had a higher thiamine content (7.1 µg./g.) than soft wheats (6.1 µg./g.). Nordgren and Andrews (11) observed that location had a much greater effect than variety on the thiamine content of spring wheat, and that the thiamine content was correlated with the ash content. Johannson and Rich (9) found a large variability (2.9 to 8.0 µg/g.) in the thiamine content of wheat and suggest that this variation may be due to soil composition, climatic conditions, and variety, while Whiteside and Jackson (18) reported a significant difference in the thiamine content of different varieties of spring wheat and stated that location and year also had a significant effect. Hoffer, Alcock, and Geddes (6) analyzed Canadian spring wheats from

three provinces and reported a range in thiamine from 2.9 to 6.3 μg./g., with an average of 4.56 μg./g. They found a significant positive correlation between thiamine content and protein. Teply, Strong, and Elvehjem (16) have reported that the environmental differences under which wheat is grown influence the niacin, pantothenic acid, and pyridoxine content, but that the effect on each of the three vitamins was not in the same order. Knox, Heller, and Sieglinger (10) found that sorghum grains vary but little in riboflavin and pantothenic acid, while niacin varied as much as 100%. Hunt, Ditzler, and Bethke (8), and Ditzler, Hunt, and Bethke (4) have shown that both hereditary and environmental factors affected the niacin and pantothenic acid content of corn hybrids and some related inbred lines. Pantothenic acid was much more subject to environmental influences than was niacin.

Long range cultural experiments inaugurated at the Ohio Agricultural Experiment Station in 1893 and in 1937 offered an excellent opportunity to study the effect of soil treatment with fertilizers and lime on the vitamin B-complex content of wheat, corn, and oats. If soil treatment affects the vitamin B-complex content of grains the fact should become evident in these types of experiments.

Materials and Methods

The types of experiments from which samples were gathered for assay were as follows:

(1) *Five-Year Rotation Fertility.* This experiment was inaugurated in 1893. The order of crops was corn, wheat, oats, and two years of forage crops. Samples were assayed from two crops of each of the cereal grains. The fertilizer treatments for each grain are shown in

TABLE I

VARIANCE FOR THIAMINE, RIBOFLAVIN, NIACIN, AND PANTOTHENIC ACID
CONTENTS OF WHEAT (FIVE-YEAR ROTATION EXPERIMENT)

Source of variance	Degrees of freedom	Thiamine	Riboflavin	Niacin	Pantothenic acid
Year	1	31.38**	48.19**	3.38	0.08
Lime	1	35.50**	.13	22.04**	18.85**
Fertilizer	8	3.75*	.42	6.71*	4.56*
Year × lime	1		1.65	1.00	3.92
Year × fertilizer	8	3.00	1.61	7.22**	2.68
Lime × fertilizer	8	1.63	.38	.90	2.42
Error	8	.08	.0069	2.16	.75
Total	35				

* Significant.
** Highly significant.

TABLE II

EFFECT OF FERTILIZER TREATMENT ON THE THIAMINE, RIBOFLAVIN, NIACIN, AND PANTOTHENIC ACID CONTENT OF WHEAT FIVE YEAR ROTATION (MICROGRAMS PER GRAM)

Plot No.	Fertilizer treatment—pounds per acre	Thiamine 1945	Thiamine 1946	Riboflavin 1945	Riboflavin 1946	Niacin 1945	Niacin 1946	Pantothenic acid 1945	Pantothenic acid 1946
2	Superphosphate, 160	3.9	3.9	0.76	0.91	41.3	40.3	9.1	8.6
	Superphosphate, 160+lime	4.5	3.9	0.77	0.93	48.3	41.1	11.3	7.7
3	Muriate of potash, 100	3.1	2.6	0.74	0.96	44.0	39.5	9.2	10.3
	Muriate of potash, 100+lime	3.4	3.7	0.81	0.87	45.3	42.7	11.8	9.5
4	No treatment (check plot)	2.6	3.6	0.82	0.89	42.0	39.7	8.9	9.3
	No treatment (check plot)+lime	3.4	4.2	0.63	0.89	46.3	43.8	13.5	13.6
5	Nitrate of soda, 160	2.8	3.5	0.71	0.97	46.3	42.8	8.5	10.6
	Nitrate of soda, 160+lime	3.7	4.7	0.84	0.78	48.0	47.4	10.6	11.4
6	Superphosphate, 160; nitrate of soda, 160	2.9	3.8	0.77	0.89	42.0	44.7	8.5	10.0
	Superphosphate, 160; nitrate of soda, 160+lime	3.0	4.1	0.88	0.83	44.7	45.3	9.2	8.8
8	Superphosphate, 160; muriate of potash, 100	2.2	3.4	0.71	0.91	36.3	41.5	10.6	9.2
	Superphosphate, 160; muriate of potash, 100+lime	3.5	4.2	0.73	0.94	40.3	41.0	10.1	10.4
9	Muriate of potash, 100; nitrate of soda, 160	3.1	3.9	0.67	1.02	39.3	43.9	9.5	12.9
	Muriate of potash, 100; nitrate of soda, 160+lime	3.6	4.6	0.81	1.03	38.7	44.4	11.9	13.8
11	Superphosphate, 160; muriate of potash, 100; nitrate of soda, 160	3.4	3.7	0.67	1.03	38.3	43.7	10.6	8.7
	Superphosphate, 160; muriate of potash, 100; nitrate of soda, 160+lime	3.8	4.3	0.83	0.99	41.7	46.2	11.5	10.8
17	Superphosphate, 160; muriate of potash, 100; nitrate of soda, 80	3.3	3.9	0.70	1.02	37.0	42.9	9.8	10.9
	Superphosphate, 160; muriate of potash, 80+lime	3.7	3.4	0.66	1.11	38.3	43.4	11.2	10.7
	Average	3.3	3.9	0.75	0.94	42.1	43.0	10.3	10.4
Minimum significant difference—Plot means		0.41		0.12		2.13		1.26	
Yearly means		0.19		0.18		0.99		0.58	

TABLE III

EFFECT OF FERTILIZER TREATMENT ON THE THIAMINE, RIBOFLAVIN, NIACIN, AND PANTOTHENIC ACID CONTENT OF CORN FIVE-YEAR ROTATION EXPERIMENT (MICROGRAMS PER GRAM)

Plot No.	Fertilizer treatment—pounds per acre	Thiamine		Riboflavin		Niacin		Pantothenic acid	
		1944	1948	1944	1948	1944	1948	1944	1948
2	Superphosphate, 80	3.2	5.3	0.97	1.14	20.7	24.8	5.7	8.6
	Superphosphate, 80+lime	4.2	6.6	0.83	1.05	20.7	24.8	5.3	7.8
3	Muriate of potash, 80	2.9	3.8	0.87	1.19	21.2	21.9	5.2	8.8
	Muriate of potash, 80+lime	3.1	5.2	0.77	1.22	20.7	23.3	4.7	6.0
4	No treatment—check plot	3.0	5.9	1.15	1.37	21.5	23.7	7.0	8.9
	No treatment—check plot+lime	4.0	6.5	1.05	1.09	21.5	25.3	5.2	10.2
5	Nitrate of soda, 160	3.6	4.9	1.08	1.09	21.7	23.7	6.2	6.7
	Nitrate of soda, 160+lime	3.9	5.3	1.00	1.10	21.7	24.7	5.6	5.9
7	No treatment—check plot		3.7		1.20		22.7		6.6
	No treatment—check plot+lime		4.8		1.09		23.8		8.4
8	Superphosphate, 80; muriate of potash, 80		5.5		0.98		24.9		5.2
	Superphosphate, 80; muriate of potash, 80+lime		6.9		1.05		25.0		4.6
9	Muriate of potash, 80; nitrate of soda, 160	3.3	5.4	0.98	0.99	19.2	23.9	5.6	7.9
	Muriate of potash, 80; nitrate of soda, 160+lime	4.3	6.4	0.93	1.03	20.7	24.5	5.2	4.1
10	No treatment—check plot		4.3		1.41		24.6		4.8
	No treatment—check plot+lime		5.2		1.06		25.5		7.7
11	Superphosphate, 160; muriate of potash, 100; nitrate of soda, 160	3.7	4.8	1.08	1.05	20.7	24.9	6.0	5.3
	Superphosphate, 160; muriate of potash, 100; nitrate of soda, 160+lime	4.6	6.3	1.00	1.09	25.0	23.9	5.3	7.3
17	Superphosphate, 80; muriate of potash, 80; nitrate of soda, 160	3.8	4.1	1.03	0.99	20.7	26.0	6.0	7.0
	Superphosphate, 80; muriate of potash, 80; nitrate of soda, 160+lime	4.7	4.9	1.08	1.04	21.7	25.7	4.9	8.6
	Average	3.7	5.3	0.99	1.12	21.3	24.4	5.6	6.8
Minimum significance difference—Plot means		0.38		0.14		1.8		1.8	
Yearly means		0.06		0.07		0.9		0.9	

TABLE IV

Effect of Fertilizer Treatment on the Thiamine, Riboflavin, Niacin, and Pantothenic Acid Content of Oats

Five Year Rotation (Micrograms per gram)

Plot No.	Fertilizer treatment—pounds per acre	Thiamine 1944	Thiamine 1945	Riboflavin 1944	Riboflavin 1945	Niacin 1944	Niacin 1945	Pantothenic acid 1944	Pantothenic acid 1945
2	Superphosphate, 80	4.0	4.0	1.37	1.18	14.3	7.3	7.8	7.0
	Superphosphate, 80+lime	4.5	5.3	1.25	1.25	10.3	6.6	7.3	8.5
3	Muriate of potash, 80	3.6	4.4	1.33	1.10	13.2	6.4	7.2	5.8
	Muriate of potash, 80+lime	3.8	4.7	1.30	1.28	10.2	6.6	6.6	8.2
4	No treatment (check plot)	3.5	4.4	1.47	1.12	13.4	6.4	6.8	5.7
	No treatment (check plot)+lime	5.1	4.9	1.37	1.19	9.8	7.6	7.2	5.2
5	Nitrate of soda, 160	4.1	5.6	1.32	1.27	13.9	6.5	8.2	5.4
	Nitrate of soda, 160+lime	5.3	6.2	1.25	1.37	10.4	6.9	6.9	4.8
6	Superphosphate, 80; nitrate of soda, 160		5.6		1.24		6.3		4.1
	Superphosphate, 80; nitrate of soda, 160+lime		5.8		1.20		7.1		7.6
8	Superphosphate, 80; muriate of potash, 80		4.0		1.13		6.6		5.8
	Superphosphate, 80; muriate of potash, 80+lime		5.8		1.32		6.8		5.2
9	Muriate of potash, 80; nitrate of soda, 160	4.0	5.0	1.45	1.38	12.0	6.7	7.5	7.0
	Muriate of potash, 80; nitrate of soda, 160+lime	4.6	5.6	1.25	1.44	10.0	6.9	7.3	7.4
11	Superphosphate, 80; muriate of potash, 80; nitrate of soda, 160	4.0	4.2	1.28	1.30	13.2	6.6	7.7	5.8
	Superphosphate, 80; muriate of potash, 80; nitrate of soda, 160+lime	4.2	4.4	1.16	1.39	11.7	7.1	7.9	7.8
17	Superphosphate, 160; muriate of potash, 80; nitrate of soda, 80	3.5	4.2	1.22	1.16	13.0	6.6	7.0	7.5
	Superphosphate, 160; muriate of potash, 80; nitrate of soda, 80+lime	4.5	5.6	1.05	1.09	11.2	6.4	6.4	8.2
	Average	4.2	5.1	1.29	1.24	11.9	6.7	7.3	6.2
Minimum significant difference—Plot means		0.5		0.04		0.8		1.0	
Yearly means		0.24		0.04		0.4		0.5	

Tables II, III, and IV.   Where lime is indicated, one-half of each plot was limed to pH 7.0.

(2) *Continuous Culture.*   Corn, wheat, and oats were grown continuously on separate but adjacent plots since 1893.   One-half of each plot was limed to pH 7.0 on all grains until 1934; since that time the entire plots on which wheat was grown have been limed.   The fertilizer treatments for each grain are shown in Tables VI, VII, and VIII. Samples assayed included wheat and oats grown during two years, and a small number of samples of corn grown during three years.

The soil underlying the plots used in the above experiments is Wooster silt loam.

(3) *Rate of Fertilization.*   This experiment was inaugurated in 1937.   The rotation of crops grown was corn, wheat, and two years of alfalfa.   Samples of wheat grown during four years were assayed. All plots received a basic treatment of lime to pH 7.0.   The fertilizer treatment is shown in Table IX.   The underlying soil is Canfield silt loam.

The same varieties of wheat and oats were grown throughout the experiments.   The same corn hybrid was used throughout the experiments with the exception of one year of the five-year rotation fertility experiment; this fact will be treated more fully in the discussion of results.

All samples were finely ground in a Wiley mill and stored in tightly stoppered glass containers in a dark room until assayed.   The assays for niacin and pantothenic acid were made as outlined in a previous publication, Hunt *et al.* (8), using *Lactobacillus arabinosus* 17-5 as the test organism.

The samples for riboflavin assay were prepared according to the procedure of Cooperman and Elvehjem (3) and determined according to the method of Snell and Strong (1939), using *Lactobacillus casei E* as the test organism.

The method used for thiamine followed closely that of Sarett and Cheldelin (13), using *Lactobacillus fermentum 36* as the test organism. The samples were subjected to enzymatic hydrolysis.   Bacterial growth at the end of 16 to 18 hours was measured as turbidity, which was read directly on the galvanometer scale of the Coleman spectrophotometer, at a wave length of 540 mμ.   The scale was first standardized with distilled water to read 100.   Readings on duplicates of three sample aliquots were made in matched tubes.   The density L ($L = 2 - \log G$) was calculated from the galvanometer reading G.   To obtain a standard curve, L values were plotted against concentrations of thiamine in aliquots of a standard solution.

TABLE V

THE RELATIONSHIP BETWEEN WEIGHT OF OATS AND PERCENT HULLS, AND NIACIN CONTENT OF OATS, GROATS, AND HULLS

Oats 1944—Dry weather

Plot No.	Fertilizer treatment—pounds per acre	Weight per bu.	Groats	Hulls	Niacin—μg./g. Oat grains	Niacin—μg./g. Groats	Niacin—μg./g. Hulls
		lb.	pct.	pct.			
2	Superphosphate, 80	23	60.2	39.8	14.3	12.0	18.2
	Superphosphate, 80+lime	30	66.6	33.4	10.3	10.6	11.4
3	Muriate of potash, 80	25	63.4	36.6	13.2	11.7	16.3
	Muriate of potash, 80+lime	30	66.5	33.5	10.2	9.7	11.2
4	No treatment (check plot)	23	62.8	37.2	13.4	12.0	17.2
	No treatment (check plot)+lime	28	67.4	32.6	9.8	9.7	10.5
5	Nitrate of soda, 160	25	62.3	37.7	13.9	10.5	17.5
	Nitrate of soda, 160+lime	28	66.6	33.4	10.4	8.0	11.2
9	Muriate of potash, 80; nitrate of soda, 160	26	61.9	38.1	12.0	10.2	13.7
	Muriate of potash, 80; nitrate of soda, 160+lime	29	69.0	31.0	10.0	8.8	9.9
11	Superphosphate, 80; muriate of potash, 80; nitrate of soda, 160	27	66.6	33.4	13.2	10.5	20.5
	Superphosphate, 80; muriate of potash, 80; nitrate of soda, 160+lime	30	68.7	31.3	11.7	9.5	16.2
17	Superphosphate, 160; muriate of potash, 80; nitrate of soda, 80	26	64.7	35.3	13.0	10.7	19.0
	Superphosphate, 160; muriate of potash, 80; nitrate of soda, 80+lime	28	66.5	33.5	11.2	9.5	15.3
	Average—unlimed	25	63.1	36.9	13.0	11.2	17.6
	Average—limed	29	67.3	32.7	10.4	9.4	12.4

TABLE V—*Cont.*

Oats 1945—Normal weather

Plot No.	Fertilizer treatment—pounds per acre	Weight per bu.	Groats	Hulls	Niacin—μg./g.		
					Oat Grains	Groats	Hulls
		lb.	*pct.*	*pct.*			
2	Fertilizer treatment, same as above	31.0 / 31.0	65.9 / 65.9	34.1 / 34.1	7.3 / 6.6	6.4 / 6.9	6.4 / 7.4
3	Fertilizer treatment, same as above	31.5 / 28.5	67.4 / 63.2	32.6 / 36.8	6.4 / 6.6	7.1 / 6.4	7.2 / 8.0
4	Fertilizer treatment, same as above	30.0 / 30.0	67.6 / 66.7	32.4 / 33.3	6.4 / 7.6	6.4 / 7.1	6.1 / 7.3
5	Fertilizer treatment, same as above	31.5 / 30.0	67.9 / 62.7	32.1 / 37.3	6.5 / 6.9	6.6 / 7.3	6.5 / 8.1
9	Fertilizer treatment, same as above	29.5 / 29.0	66.4 / 63.4	33.6 / 36.6	6.7 / 6.9	6.7 / 7.5	7.8 / 8.6
11	Fertilizer treatment, same as above	30.0 / 29.5	68.8 / 67.8	31.2 / 32.2	6.6 / 7.1	5.8 / 7.8	5.8 / 8.7
17	Fertilizer treatment, same as above	31.0 / 30.5	67.8 / 68.4	32.2 / 31.6	6.6 / 6.4	7.4 / 7.2	6.9 / 5.8
	Average—unlimed / Average—limed	30.6 / 29.8	67.4 / 65.4	32.6 / 34.6	6.7 / 6.8	6.7 / 7.1	6.6 / 7.7

TABLE VI

EFFECT OF FERTILIZER TREATMENTS ON THE THIAMINE, RIBOFLAVIN, NIACIN, AND PANTOTHENIC ACID CONTENT OF WHEAT CONTINUOUS CULTURE (MICROGRAMS PER GRAM)

Plot No.	Fertilizer treatment—pounds per acre	Thiamine		Riboflavin		Niacin		Pantothenic acid	
		1947	1948	1947	1948	1947	1948	1947	1948
1	No fertilizer (check plot)	3.0	4.4	1.13	1.23	46.3	48.7	13.1	13.4
	No fertilizer (check plot)+lime (before 1934)	3.5	4.8	1.15	1.32	42.5	50.6	13.4	13.4
2	Superphosphate, 128; muriate of potash, 100; nitrate of soda, 160	3.3	4.7	1.25	1.16	42.7	45.1	11.8	11.7
	Superphosphate, 128; muriate of potash, 100; nitrate of soda, 160+lime (before 1934)	3.6	4.5	1.15	1.22	43.3	45.7	10.5	11.2
3	Superphosphate, 128; muriate of potash, 100	3.5	4.8	0.95	1.26	41.8	45.7	10.2	12.2
	Superphosphate, 128; muriate of potash, 100+lime (before 1934)	3.7	5.1	1.08	1.31	38.8	45.0	10.2	13.4
4	No fertilizer (check plot)	3.5	4.6	0.93	1.04	44.3	45.3	13.2	13.9
	No fertilizer (check plot)+lime (before 1934)	3.6	4.7	1.21	1.19	40.5	48.7	11.4	13.4
5	Barnyard manure—2½ tons		4.5		1.21		47.7		13.2
	Barnyard manure—2½ tons+lime (before 1934)		5.1		1.17		49.6		13.8
6	Barnyard manure—5 tons	3.8	4.9	1.08	1.23	54.3	47.6	12.8	13.9
	Barnyard manure—5 tons+lime (before 1934)	3.7	4.7	1.20	1.10	50.7	45.3	12.1	13.0
7	No fertilizer (check plot)	3.2	4.6	1.01	1.32	48.2	45.3	12.1	13.4
	No fertilizer (check plot)+lime (before 1934)	3.8	4.8	1.00	1.26	46.1	48.1	12.5	13.9
8	Superphosphate, 256; muriate of potash, 200; nitrate of soda, 320	3.6	4.8	1.06	1.22	55.1	47.3	12.9	13.1
	Superphosphate, 256; muriate of potash, 200; nitrate of soda, 320+lime (before 1934)	3.4	4.9	1.04	1.11	44.1	47.6	12.5	12.5
9	Superphosphate, 256; muriate of potash, 200	3.7	4.9	1.15	1.18	50.2	43.7	12.3	12.5
	Superphosphate, 256; muriate of potash, 200+lime (before 1934)	3.3	4.6	1.03	1.29	45.8	43.4	11.6	12.7
10	No fertilizer (check plot)	3.3	4.4	1.00	1.14	53.6	48.4	13.5	14.4
	No fertilizer (check plot)+lime (before 1934)	3.3	3.9	1.09	1.35	46.4	47.3	12.1	13.1
	Average	3.5	4.7	1.08	1.21	46.4	46.8	12.1	13.1
	Minimum significant difference—Plot means	0.35		0.14		4.00		0.80	
	Yearly means	0.14		0.06		1.86		0.40	

TABLE VII

The Effect of Fertilizers on the Thiamine, Riboflavin, Niacin, and Pantothenic Acid Content of Corn Continuous Culture (Micrograms per Gram)

Plot No.	Fertilizer treatment (lbs./acre)	Thiamine			Riboflavin			Niacin			Pantothenic acid		
		1945	1946	1947	1945	1946	1947	1945	1946	1947	1945	1946	1947
1	No fertilizer (check plot)	3.4	3.7	3.8	0.88	0.97	1.13	23.8	27.1	28.8	6.8	5.7	5.8
	No fertilizer (check plot)+lime	3.4	3.4	3.5	0.88	0.95	0.87	21.0	25.6	26.0	5.5	5.1	6.1
8	Superphosphate, 256; muriate of potash, 200; nitrate of soda, 320	3.6	4.5	3.7	0.76	0.88	0.94	21.7	25.4	25.5	5.5	4.5	6.2
	Superphosphate, 256; muriate of potash, 200; nitrate of soda, 320+lime	3.8	4.2	3.5	0.82	0.88	0.92	21.0	25.5	28.0	6.8	5.3	5.9
	Average	3.6	4.0	3.6	0.84	0.92	0.97	21.9	25.9	27.1	6.2	5.2	6.0
	Minimum significant difference—Plot means	0.06			0.07			1.30			1.04		
	Yearly means	0.07			0.09			1.60			1.29		

TABLE VIII

EFFECT OF FERTILIZER TREATMENT ON THE THIAMINE, RIBOFLAVIN, NIACIN, AND PANTOTHENIC ACID CONTENT OF OATS
CONTINUOUS CULTURE (MICROGRAMS PER GRAM)

Plot No.	Fertilizer treatment—pounds per acre	Thiamine		Riboflavin		Niacin		Pantothenic acid	
		1947	1948	1947	1948	1947	1948	1947	1948
1	No fertilizer—check plot	5.0	5.8	3.73	0.97	10.5	8.5	13.7	10.1
	No fertilizer—check plot+lime	5.9	6.3	2.98	1.02	9.4	9.0	15.7	11.1
2	Superphosphate, 128; muriate of potash, 100; nitrate of soda, 160	5.6	6.0	2.49	0.85	8.1	8.9	12.5	10.9
	Superphosphate, 128; muriate of potash, 100; nitrate of soda, 160+lime	5.5	6.1	2.92	0.88	9.5	8.9	13.1	9.2
3	Superphosphate, 128; muriate of potash, 100	4.0	5.4	2.40	0.88	8.1	8.6	10.1	10.9
	Superphosphate, 128; muriate of potash, 100+lime	4.7	5.7	2.71	0.90	8.9	8.2	12.6	10.2
4	No fertilizer—check plot	5.4	6.1	3.72	0.99	10.1	8.2	14.1	9.4
	No fertilizer—check plot+lime	5.1	6.2	3.07	0.91	9.9	8.9	11.1	10.0
5	Barnyard manure—2.5 tons	5.5	5.4	2.95	0.84	8.1	8.3	9.8	9.6
	Barnyard manure—2.5 tons+lime	6.1	5.7	2.62	0.84	9.2	8.6	10.3	10.3
6	Barnyard manure—5 tons	4.0	5.7	2.24	0.92	9.1	8.5	10.0	10.5
	Barnyard manure—5 tons+lime	5.5	5.5	2.62	0.85	9.1	8.9	9.9	8.8
7	No fertilizer—check plot	5.2	5.8	2.72	0.93	9.0	8.6	9.5	9.9
	No fertilizer—check plot+lime	4.7	6.0	2.62	0.97	9.2	8.9	10.0	9.9
8	Superphosphate, 256; muriate of potash, 200; nitrate of soda, 320	5.6	6.5	2.16	0.95	8.1	8.5	9.1	9.2
	Superphosphate, 256; muriate of potash, 200; nitrate of soda, 320+lime	6.1	6.0	2.13	1.05	9.8	7.5	9.7	11.8
9	Superphosphate, 256; muriate of potash, 200	4.1	4.1	2.42	0.91	7.7	8.2	7.2	6.6
	Superphosphate, 256; muriate of potash, 200+lime	4.2	4.5	2.62	0.95	8.8	8.7	8.4	12.6
10	No fertilizer—check plot	4.5	6.1	3.09	1.03	9.5	8.4	10.4	12.0
	No fertilizer—check plot+lime	4.3	6.1	2.77	1.14	9.3	8.8	10.2	10.7
	Average	5.1	5.8	2.75	0.94	9.1	8.6	10.9	10.2
	Minimum significant difference—Plot means	0.30		0.30		0.89		1.87	
	Yearly means	0.14		0.14		0.40		0.85	

TABLE IX

EFFECT OF RATE OF FERTILIZATION ON THE VITAMIN B-COMPLEX OF WHEAT
(MICROGRAMS PER GRAM)

Plot No.	1	3	6	9	
Fertilizer treatment	None +lime	200#2-12-6 +lime	500#2-12-6 +lime	1000#2-12-6 +lime	Av.
lbs./acre					
Thiamine					
1944	3.2	3.4	3.8	3.4	3.5
1945	5.4	5.1	5.3	5.3	5.3
1946	4.7	4.9	4.8	5.0	4.9
1947	4.2	3.8	3.6	3.2	3.7
Av.	4.4	4.3	4.4	4.2	4.3
Riboflavin					
1944	1.04	0.94	1.13	0.82	0.98
1945	1.16	1.28	1.18	1.21	1.21
1946	.89	.96	1.15	.91	.98
1947	1.09	1.08	1.10	1.15	1.10
Av.	1.05	1.07	1.23	1.02	1.08
Niacin					
1944	60.2	57.0	52.0	51.2	55.1
1945	58.7	60.3	59.7	56.3	58.8
1946	43.8	48.5	49.8	51.8	48.5
1947	51.7	46.3	44.3	44.0	46.6
Av.	53.6	53.0	51.5	50.8	52.2
Pantothenic acid					
1944	12.0	13.2	12.8	11.8	12.8
1945	12.3	12.3	10.9	10.5	11.5
1946	10.5	10.3	11.7	10.4	10.7
1947	14.4	12.9	11.5	12.0	12.7
Av.	12.3	12.2	11.7	11.2	11.8

Minimum significant difference—yearly means: Thiamine, 0.43, Riboflavin, 0.14, Niacin, 5.53, Pantothenic Acid, 1.35.

A reference sample was used in each set of assays and for each vitamin. If the assay of the reference sample was within 10% of its known vitamin content, the assay values of the unknown samples were accepted.

All of the data were subjected to statistical analysis. Interpretations of results are based on statistically significant differences in vitamin content.

Results and Discussion

Five-Year Rotation Fertility Experiment. The fertilizer treatments consisted of nitrogen, phosphorus, and potash additions and various combinations of these elements, all with and without lime and no treatment. Each plot received a different fertilizer treatment. Analysis of variance was made on the data. As an illustration, Table I shows the variance for the vitamins of wheat. Each vitamin was tested for variance due to year, lime, fertilizer, and interactions of each

with the other.   In the cases where the data of only one year were
available from a given plot, that plot was omitted from the analysis of
variance test.

Wheat. The data for wheat are presented in Table II. The
results show that season (i.e., the year the samples were grown) in-
fluenced the thiamine and riboflavin contents. The wheat grown in
1946 had significantly higher thiamine and riboflavin contents than
that grown in 1945. This was true irrespective of the fertilizer
treatment of the soil. Season had no effect on the niacin and panto-
thenic acid contents of wheat. The most outstanding and at the same
time constant result was a higher thiamine, niacin, and pantothenic
acid content produced by the application of lime. This effect (Table
II) was highly significant for both years. The effects of fertilizers on
thiamine were variable. The data indicate that the applications of
superphosphate and potash as single treatments resulted in a slight
increase and decrease, respectively, in thiamine content. This was
true for both years of the study. Nitrate fertilizer alone significantly
increased the niacin content of wheat, and no fertilizer treatment
(check plot), as well as nitrate and potash in combination, produced
wheat of the highest pantothenic acid content.

Hunt (7) reported that liming of the soil increased the phosphorus
and magnesium contents of wheat. The data in this study show that
liming of the soil increased the thiamine content, suggesting that there
may be a close association between phosphorus and magnesium and
the thiamine content of wheat. Since no mineral analyses were made
on corn or oats in this or other studies, it is not known whether or not
this association might occur in these cereals.

Corn. Season had a significant effect on the thiamine, riboflavin,
niacin, and pantothenic acid contents of corn (Table III). The 1948
crop was higher in all four vitamins than the 1944 crop. The hybrid
Ohio W17 was grown in 1944 and Ohio W36 was grown in 1948. In
view of this fact, differences due to season cannot be clearly determined
since the two hybrids may have responded to the seasonal variations in
a different manner; however, the inference is that season had a signifi-
cant effect.

Weather records show that 1944 was very dry during the growing
season, while 1948 was termed a normal growing period with a normal
rainfall. As with wheat, lime increased the thiamine content signifi-
cantly while potash decreased the thiamine content significantly.
Lime decreased riboflavin and had no effect on niacin and pantothenic
acid.

Oats. The data on oats show further evidence that season affected
the thiamine content of the cereals studied (Table IV). The 1945

crop was significantly higher in thiamine than the 1944 crop.   Liming of the soil increased the thiamine content.   Nitrate fertilizer alone increased the thiamine content of oats.   Lime decreased riboflavin in 1944 and increased it in 1945; these effects were slight, quantitatively, but statistically significant because of consistency throughout the plots.

The 1944 crop of oats was higher in niacin than the 1945 crop by approximately 75%.   This difference was highly significant.   In 1944, the limed plots yielded oats consistently lower in niacin than that from the unlimed plots; in 1945, the effect of lime was not consistent. The fertilizer treatments were the same for both years, so that the differences in the niacin content and in the effect of lime could not be accounted for on the basis of fertilizer.   In search of a possible explanation, other factors were investigated.   Weather records showed that 1944 was a dry year and 1945 had normal rainfall.   Weight per bushel and hull content of oats were determined (Table V).   In 1944, liming the soil was shown to be associated not only with lower niacin but also with higher weight per bushel and obviously lower hull content. The niacin content of hulls was higher than that of groats.   In 1945, the effect of lime was not consistent for niacin, weight per bushel, or hull content.   From the foregoing facts, it was concluded that factors which increase hull content of oats also increase the niacin content. The action of lime during a dry year had a decreasing effect on niacin with related effects on weight per bushel and hull content.   It is interesting to note that differences between the hull and niacin content of oats when grown on the limed and unlimed plots were less where a complete fertilizer (NPK) was used than where a single fertilizer (N or P or K) was applied to the soil (see Table V).   This appears to apply only under extreme weather (dry) conditions.

The fact that liming the soil increased the thiamine content of all three cereals studied would lead to a general statement that liming the type of soil on which the crops were grown for this study would increase the thiamine content of wheat, corn, and oats, irrespective of season and the fertilizer applied to the soil.

Continuous Culture Experiment.   The fertilizer treatments consisted of phosphate and potash in combination, complete, barnyard manure, and no treatment.   In wheat and oats, samples from two or more replications of each fertilizer treatment were assayed, with the exception of barnyard manure on wheat in 1947, of which one sample only was assayed.   Analysis of variance was performed to test variance due to year, lime, and plot, and interactions of each with the other. Variance due to plot may have been the result of fertilizer treatment as well as the variance between replications in the same field.   In order to determine the significance of differences due to fertilizer, group

comparisons (t values) were made on the grouped samples receiving a given fertilizer with other groups on different fertilizer treatments.

Wheat. As in the five-year rotation fertility experiment, season affected the thiamine and riboflavin, and, in addition, the pantothenic acid content (Table VI). The wheat grown in 1948 (normal weather) had higher thiamine, riboflavin, and pantothenic acid contents than wheat grown in 1947 (high rainfall in April, May, and June). Comparison of fertilizer treatments for both years shows that the check plot (no fertilizer) produced wheat of the highest pantothenic acid content. As previously noted, one-half of each plot was limed until 1934, after which the entire plot was limed. At the time the samples were collected, therefore, both ends of the plots were receiving the same lime treatment. Any variations in the vitamin content of wheat grown on the two ends of the plots, as noted, may be due to a residual effect of liming previous to 1934 (Table VI).

Corn. The data in Table VII show that the niacin content of corn was influenced by season. No other effects of fertilizers, including lime, were observed. This does not agree with the results obtained in the five-year rotation experiment.

Oats. Assay results (Table VIII) show that the thiamine content of oats is affected by the season and that complete fertilizer (NPK) barnyard manure, and no fertilizer (check plot) produced oats of higher thiamine content than phosphorus and potash in combination. Liming the soil increased the thiamine content of oats but the effect was not as significant as that found in the five-year rotation experiment. The effect of liming on the niacin content of oats was not so discernible as in the five-year rotation experiment. A difference between a dry and wet and normal season may be the cause of this difference.

The riboflavin and niacin contents of this cereal were influenced by year (Table VIII). The oats grown in 1947 were much higher in riboflavin and slightly higher in niacin than that grown in 1948. This difference in effect of season appears to be due to the seasonal (weather) effect on that part of the grains in which the greater portion of the vitamin was or is being stored. Due to excess rainfall, the 1947 crop was very light in weight (about 20 pounds per bushel), which would indicate a high hull content. As was stated previously, light weight, high hull oats grown in the dry year, 1944, on the five-year rotation fertility experiment, were found to be correlated with a considerable increase in niacin. In the continuous culture experiment, the variation due to season was not nearly so marked.

These differences in magnitude of the effects of lime and season may have been due to type of experiment.

Rate of Fertilization Experiment. Wheat. The results are shown in Table IX. As in the other two experiments, the results show that season had a significant effect on the thiamine, riboflavin, niacin, and pantothenic acid contents of wheat. There was no consistent effect of extreme seasons (excessive rainfall or dryness) on the vitamins.

The rate of fertilizer application to the soil in this experiment had no significant effect on the vitamin B-complex content. It is also noted that there was no difference between the vitamin content of wheat from the check and fertilized plots.

The wheat grown on the plots in this experiment (of a more recent origin) had higher average thiamine, riboflavin, niacin, and pantothenic acid contents than the wheat grown on the five-year rotation fertility experiment, and the niacin content of the continuous culture experiment. This difference may be accounted for by the type of experiment and the length of time the experiment has been in operation.

The effect of single fertilizer ingredients and combinations of single fertilizer ingredients, as reported in this study, have been stated with caution due to lack of replication of plots in the five-year rotation experiment. However, it was hoped that year duplication would suffice in a manner to make the data valuable. The statistical analyses have indicated the results. Replicate plots in the continuous culture experiment showed some variation. The fact that all cereals from all experiments were not available the same season (year) no doubt caused variations in vitamin content which cannot be properly evaluated statistically. It should be noted, however, that the results, while not always highly significant, are good indications of the effect of fertilizers on the vitamin B-complex of cereals as stated.

Literature Cited

1. AMES, J. W., and BOLTZ, G. E.   Relation of phosphorus and nitrogen in soil to the composition of wheat.   Ohio Agr. Exp. Sta. Bulletin No. 318 (1917).
2. AMES, JOHN W., BOLTZ, G. E., and STENIUS, J. A.   Effect of fertilizers on the physical and chemical properties of wheat.   Ohio Agr. Exp. Sta. Bulletin No. 243 (1912).
3. COOPERMAN, J. M., and ELVEHJEM, C. A.   The B vitamin content of groats and rolled oats.   J. Nutrition 27: 329–333 (1944).
4. DITZLER, L., HUNT, C. H., and BETHKE, R. M.   Effect of heredity on the niacin and pantothenic acid content of corn.   Cereal Chem. 25: 273–279 (1948).
5. DOWNS, D. E., and CATHCART, W. H.   Thiamine content of commercial wheats of the 1940 crop.   Cereal Chem. 18: 796–801 (1941).
6. HOFFER, A., ALCOCK, A. W., and GEDDES, W. F.   The effect of variations in Canadian spring wheat on the thiamine and ash of long-extraction flours. Cereal Chem. 21: 210–221 (1944).
7. HUNT, C. H.   The influence of fertilizers on the vitamin-B content of wheat. Ohio Agr. Exp. Sta. Bulletin No. 415 (1927).
8. HUNT, CHAS. H., DITZLER, L., and BETHKE, R. M.   Niacin and pantothenic acid content of corn hybrids.   Cereal Chem. 24: 355–363 (1947).
9. JOHANNSON, H., and RICH, C. E.   The variability in the thiamine content of Western Canadian hard red spring wheat of the 1940 crop.   Cereal Chem. 19: 308–313 (1942).

10. KNOX, GRACE, HELLER, V. G., and SIEGLINGER, J. B.   Riboflavin, niacin, and pantothenic acid content of grain sorghums.   Food Research 9: 89–91 (1944).
11. NORDGREN, R., and ANDREWS, J. S.   The thiamine content of cereal grains. Cereal Chem. 18: 802–811 (1941).
12. ROWLANDS, M. J., and WILKINSON, B.   The vitamin B content of grass seed in relation to manures.   Biochem. J. 24: 199–204 (1930).
13. SARETT, H. P., and CHELDELIN, V. H.   Use of *Lactobacillus fermentum* 36 for thiamine assay.   J. Biol. Chem. 155: 153–160 (1944).
14. SCHULTZ, A. S., ATKIN, L., and FREY, C. N.   A preliminary survey of the vitamin B_1 content of American cereals.   Cereal Chem. 18: 106–113 (1941).
15. SNELL, E. E., and STRONG, F. M.   A microbiological assay for riboflavin.   Ind. and Eng. Chem. (Anal. Ed.) 11: 346–350 (1939).
16. TEPLY, L. J., STRONG, F. W., and ELVEHJEM, C. A.   Nicotinic acid, pantothenic acid, and pyridoxine in wheat and wheat products.   J. Nutrition 24: 167–174 (1942).
17. VOEGTLIN, C., and MEYERS, C. N.   The dietary deficiency of cereal foods with reference to their content in "antineuritic vitamin."   Public Health Reports XXXiii: 647–666 (1918).
18. WHITESIDE, A. G. O., and JACKSON, S. H.   The thiamine content of Canadian hard red spring wheat varieties.   Cereal Chem. 20: 540–551 (1943).

AMINO ACID CONTENT OF VARIOUS WHEAT VARIETIES
I. CYSTINE, LYSINE, METHIONINE, AND GLUTAMIC ACID [1]

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ABSTRACT

Differences in "microbiologically apparent" cystine and methionine with respect to environment were observed for several varieties of hard red winter wheat grown during one crop year.   No differences in lysine or glutamic acid with respect to environment were observed.   Likewise, no differences among wheat varieties were found for either cystine, lysine, methionine, or glutamic acid.   All values are based on total wheat protein. There was a significant difference in per cent cystine for samples grown in 1946 and 1947.   The wheat grown in 1947 contained the most cystine and also required longer mixing for optimum dough development.   Thus there may be a relationship between per cent cystine and dough mixing time as influenced by environment.   The longer mixing varieties tended to reflect a greater change in mixing requirement for a small change in cystine content. The nutritional value of wheat protein may be affected by environment due to variation in cystine and methionine content in the protein.

The protein content of wheat is variable and may be affected by various environmental factors including soil type, fertilizer treatment, and weather.   Little is known, however, concerning the relationship of these factors to the relative amino acid composition of the protein.

The development of microbiological as well as highly specific colorimetric methods for amino acid assay has made practicable the estimation of the relative proportions of specific amino acids in natural products.   Although the assessment of validity of the assays necessitates a considerable number of tests, the results lend themselves to statistical treatment.

Certain amino acids have received more attention than others. Greaves and Bracken (9) have shown that different varieties of wheat and wheat receiving various cultural treatments differ in both total sulfur and total nitrogen.   These workers found a highly significant correlation between total sulfur and total nitrogen and obtained evidence that all of the sulfur in wheat is in the organic form.   Gubler and Greaves (10) found a variation in cystine content between varieties as well as between wheats grown under varying cultural conditions. A positive correlation was observed between cystine and sulfur as well as between cystine and nitrogen content.   Approximately 50% of the total sulfur of wheat was found to be in the form of cystine.   Csonka (5) found that the protein of Marquis wheat contained 1.43% cystine while Tenmarq and Fulhio contained 1.16 and 1.18% respectively. Little work has been reported on the influence of variety and environment on the relative amino acid content of wheat proteins.

Osborne and Mendel (16) concluded that "Wheat proteins considered in their entirety are adequate for promoting normal growth if eaten in sufficient amount."   However, since the addition of animal protein to the diet greatly enhanced the value of the wheat protein for growth, a deficiency in certain amino acids was indicated.   Csonka (5) has shown that wheat protein is very deficient in tryptophan. Other essential amino acids, notably lysine and methionine, are also present in rather small quantities (3).

Although studies relating the baking quality of wheat to chemical constitution have not been fruitful, certain relationships may become apparent with further work.   The present study was initiated to obtain information on the effect of wheat species, variety, and environment on the cystine, methionine, lysine, and glutamic acid content of wheat protein.

Materials and Methods

Three sets of wheat samples selected from pure species and varieties were obtained for this study.   One set (Table I) consisted of several wheat species from the 1946 crop grown at Sacaton, Arizona.   The second set of samples (Table I) representing hard red winter wheat were composited by variety from the 1947 crop for both the Central and Southern Great Plains.   The Southern district samples were composited from equal portions of wheat grown at Amarillo, Chilli-

TABLE I

WHEAT SAMPLES SELECTED FOR MICROBIOLOGICAL ASSAY
OF THEIR CONSTITUENT AMINO ACIDS

Name	Number	%Protein[1]	
WHEAT SPECIES			
T. durum (Pentad)	C. I. 3322	17.8	
T. durum (Mindum)	C. I. 5296	15.0	
T. sphaerococcum	C. I. 4923	15.0	
T. dicoccum	C. I. 7276	18.1	
T. pyramidale	P. I. 113398	15.3	
T. orientale	P. I. 68282	15.4	
T. persicum	P. I. 115817	19.3	
T. polonicum	P. I. 127087	18.9	
HARD RED WINTER WHEAT			
Kharkof	C. I. 1442	14.9[2]	14.6[3]
Blackhull	C. I. 6251	14.9	14.2
Tenmarq	C. I. 6936	15.1	13.4
Pawnee	C. I. 11669	14.1	14.5
Comanche	C. I. 11673	14.9	15.0
Wichita	C. I. 11952	14.3	14.3
Red Chief	C. I. 12109	14.2	14.2

[1] Dry weight basis ($N \times 5.7$).
[2] Samples from the Southern District.
[3] Samples from the Central District.

cothe, and Denton, Texas, and Lawton and Stillwater, Oklahoma. The Central district samples were composited from equal portions of wheat grown at Akron, Colorado, Manhattan, and Colby, Kansas, Alliance, Lincoln, and North Platte, Nebraska. The third set of samples (see Table IV) consisted of five varieties duplicated for the 1946 and 1947 crop years. These samples were composited by variety on the basis of similar protein content from many locations throughout the state of Kansas.

Samples were prepared for assay by hydrolyzing finely ground one-gram samples (dry weight basis, weighed to $\pm$ 1 mg.) in an autoclave at 15 lbs. pressure with 25 ml. of hydrochloric acid. The hydrolysis vessels were 125 ml. Erlenmeyer flasks covered with inverted beakers. Optimum conditions for hydrolysis varied for different amino acids as is graphically illustrated in Fig. 1. For both lysine and methionine assay, hydrolysis for 10 hours with 2 N hydrochloric acid was used. For cystine, optimum results were obtained by 1-hour hydrolysis with 4 N hydrochloric acid. Results obtained for cystine using 0.5 hour hydrolysis were low. (Not shown in Fig. 1.) For glutamic acid, maximum assay values were obtained by hydrolysis for five hours with 6 N hydrochloric acid. The hydrolysates were cooled, adjusted to pH 6.8 [2] with sodium hydroxide solution, diluted to 100 ml. volume, and filtered.

[2] All pH adjustments were made using glass electrode equipment.

Microbiological methods of assay were employed throughout the study.   These methods are suitable for obtaining comparative values and for analyzing a large number of samples at one time (Baumgarten *et al.*, 3).   It is recognized that variable amounts of some amino acids, notably cystine (Riesen and coworkers, 18), are lost when heated in the presence of carbohydrates.   To minimize this error all samples being compared were chosen for similarity of protein content and were hydrolyzed and analyzed simultaneously under similar conditions. The values reported, although not absolute, are nevertheless relative and adequate for indicating differences among samples.

The organisms used in this study were *Lactobacillus arabinosus* 17-5 and *Leuconostoc mesenteroides* P-60.   These organisms were
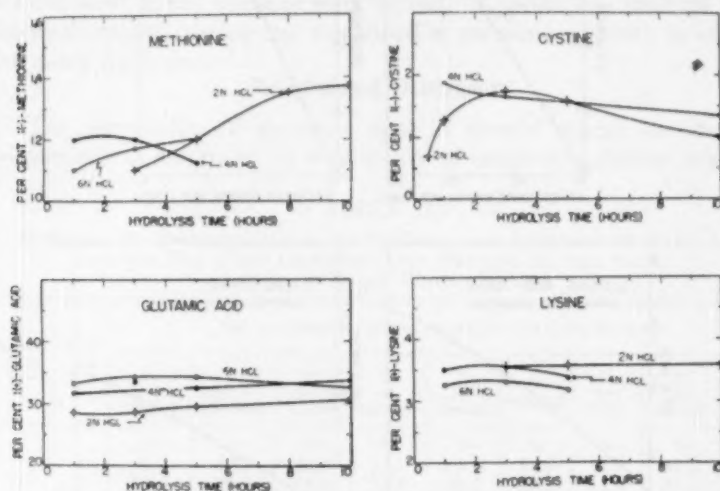
Fig. 1.   Effect of hydrolysis time and acid strength on the rate
of amino acid liberation from wheat protein.

obtained from the American type culture collection, Georgetown University School of Medicine, Washington, D. C.

The general procedure for carrying out the assays was that used by Kuiken and coworkers (13).   The tubes, after sterilization, were cooled, inoculated, and incubated at 36°C. for 72 hours.   Titrations were completed with 0.10 N sodium hydroxide in the 22 $\times$ 175 mm. incubation tubes without centrifuging.   One ml. (0.7 mg.) of brom-thymol blue in 25% ethanol was used as the indicator.   Typical standard curves are shown in Fig. 2.

Results from duplicate tubes and from all assay levels were averaged and reported as per cent of the total protein material present (dry weight basis).

The composition of the complete medium and the stock solutions used for the assay of glutamic acid with *Lactobacillus arabinosus* were essentially the same as those used by Riesen and coworkers (17).[3] The amounts of l-lysine and dl-threonine were decreased to 1 mg. per tube while 2 mg. of dl-tryptophan, 2 mg. of dl-glycine, 1 mg. of l-proline, and 1 mg. of dl-serine were added per assay tube. The quantities of purines and pyrimidine were doubled, while choline and inositol were omitted from the medium. As suggested by Lyman and
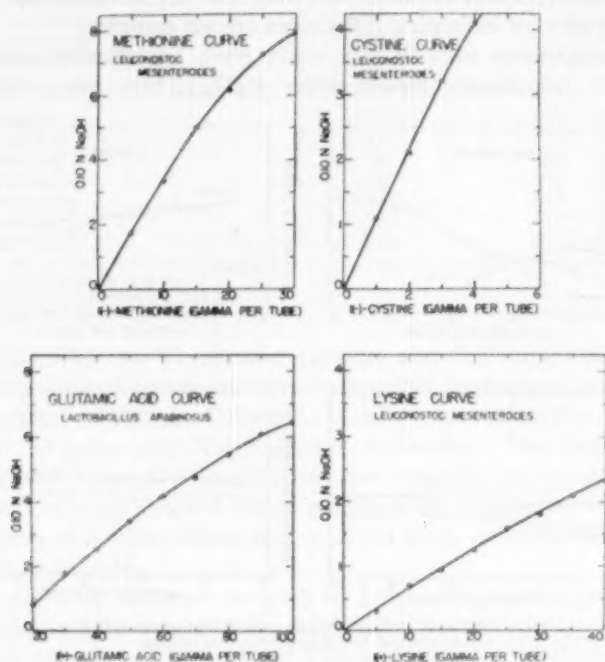
Fig. 2. Typical standard curves for the determination of methionine, cystine, glutamic acid, and lysine. Titration values are for the entire 10 ml. culture. The incubation time was 72 hours and the incubation temperature 36°C.

coworkers (14) glutamine was added to the medium in the proportion of 0.25 mg. per assay tube. Sigmoidal growth curves were obtained unless the glutamine was present in the medium.

The composition of the synthetic medium used for the assay of lysine with *Leuconostoc mesenteroides* was essentially the same as Medium C used by Dunn and coworkers (6). Hydroxyproline, norleucine, and norvaline were omitted from the medium.

[3] Certain changes in the basal medium for the assay of glutamic acid as well as for the other amino acids studied were made in order to obtain better growth curves under the conditions of the experiment and for purposes of standardization of the stock solutions.

The medium used for the microbiological assay of methionine using *Leuconostoc mesenteroides* as the test organism was a modification of that used for the determination of lysine.   Hydrogen peroxide-treated peptone was prepared as described by Lyman *et al.* (15).   The treated peptone solution was substituted for the pure amino acids with the exception of tryptophan, tyrosine, and cystine, which were added to the medium in the same quantities as used for the lysine analysis. Xanthine was included using 12 mg., per liter of basal medium.

Leuconostoc mesenteroides was selected as the most suitable organism for the microbiological assay of cystine (Riesen and coworkers, 18). The medium was the same as that used in the determination of methionine, with two exceptions.   The amounts of glucose and sodium acetate used in the medium were halved.   Cystine was omitted from the medium and methionine was added in the same quantity as used in the assay for lysine.

Results and Discussion

The percentage of glutamic acid in several wheat species was determined to ascertain the desirability of continuing similar work on

TABLE II

SUMMARY OF MICROBIOLOGICAL ASSAY DATA AND ANALYSES OF VARIANCE OF THE PER CENT GLUTAMIC ACID PRESENT IN THE TOTAL PROTEIN OF EIGHT WHEAT SPECIES

Hydrol- ysis No.	Anal- ysis No.	Per cent glutamic acid in protein ($N \times 5.7$) on dry basis								
		T. durum (Pentad)	*T. durum* (Mindum)	*T. sphaero- coccum*	*T. di- coccum*	*T. py- ramidale*	*T. orientale*	*T. per- sicum*	*T. po- lonicum*	Aver- age
		%	%	%	%	%	%	%	%	%
1	1	32.2	30.4	28.5	33.1	28.6	31.9	31.0	32.2	
2	1	37.4	35.3	28.9	32.7	29.7	29.8	31.9	34.1	
3	1	33.3	31.6	29.3	33.4	29.2	30.9	32.6	33.3	
Average		34.3	32.4	28.9	33.1	29.2	30.9	31.8	33.2	31.7
1	1	32.2	30.4	28.5	33.1	28.6	31.9	31.0	32.2	
1	2	35.7	33.2	29.8	34.3	28.7	31.9	32.3	33.3	
1	3	32.5	32.6	29.8	32.7	30.2	32.6	32.5	35.0	
Average		33.5	32.1	29.4	33.4	29.2	32.1	31.9	33.5	31.9

ANALYSES OF VARIANCE

Source of variation	Degrees of freedom	Mean squares
Species	7	11.307**
Error (One analysis on each of three hydro- lysates)	16	2.159
Species	7	9.057***
Error (Three analyses on one hydrolysate)	16	1.352

** Significance exceeds the 1% level.
*** Significance exceeds the 0.1% level.

wheat varieties. A summary of assay data and the analysis of variance are shown in Table II. No significant differences were found between single analyses on each of three different hydrolysates and triple analyses on one hydrolysate. The differences in percentages of glutamic acid present in the wheat species tested were statistically

TABLE III

SUMMARY OF MICROBIOLOGICAL ASSAY DATA AND ANALYSES OF VARIANCE
OF THE PER CENT GLUTAMIC ACID, METHIONINE, CYSTINE, AND
LYSINE PRESENT IN THE TOTAL PROTEIN OF SEVEN
COMPOSITED WHEAT VARIETIES GROWN IN
TWO WIDELY DIFFERENT AREAS

Variety	Protein[1]	Per cent of amino acid in protein ($N \times 5.7$) on dry basis[2]			
		1-Glutamic acid	1-Methionine	1-Cystine	1-Lysine
SOUTHERN DISTRICT					
	%	%	%	%	%
Kharkof	14.9	32.5	1.37	2.10	3.40
Blackhull	14.9	33.1	1.33	2.18	3.16
Tenmarq	15.1	32.9	1.36	2.13	3.30
Pawnee	14.1	32.0	1.43	2.19	3.18
Comanche	14.9	33.5	1.41	2.19	3.12
Wichita	14.3	33.3	1.37	2.14	3.16
Red Chief	14.2	33.2	1.44	2.17	3.07
Mean for Southern District	14.6	32.9	1.39	2.16	3.20
CENTRAL DISTRICT					
Kharkof	14.6	33.3	1.43	2.26	3.39
Blackhull	14.2	32.7	1.45	2.43	3.33
Tenmarq	13.4	33.5	1.54	2.47	3.41
Pawnee	14.5	32.8	1.46	2.37	3.21
Comanche	15.0	33.9	1.42	2.35	3.21
Wichita	14.3	32.8	1.44	2.36	3.15
Red Chief	14.2	32.2	1.49	2.43	3.30
Mean for Central District	14.3	33.0	1.46	2.38	3.29
Mean for both districts		33.0	1.43	2.27	3.25

ANALYSES OF VARIANCE

Source of variation	Degrees of freedom	Mean square			
		Glutamic acid	Methionine	Cystine	Lysine
Variety	6	0.007	0.003	0.007	0.037
District	1	0.001	0.040***	0.350***	0.056
V×D	6	0.005	0.003	0.005	0.008
Error	14	0.011	0.002	0.017	0.024

[1] Moisture-free basis.
[2] Mean for two separate analyses. Both hydrolysis and analyses of all varieties were performed simultaneously for each amino acid.
*** Significance exceeds the 0.1% level.

significant.   It is possible that this is the result of differences in the relative proportions of the various protein types present in the wheat.

Based on the results obtained for glutamic acid in different wheat species, the work was extended to include the determination of four amino acids in seven hard red winter wheat varieties composited from samples grown in widely different areas.   Data expressing the percentage of glutamic acid, methionine, cystine, and lysine present in the total wheat protein are recorded in Table III.   A summary of the statistical analyses of these data is also shown in Table III.   Differences in methionine, cystine, glutamic acid, and lysine among varieties were nonsignificant.   Highly significant differences, however, were found for the methionine and the cystine content of varieties grown in different districts of the Great Plains area.   The percentage of both sulfur-bearing amino acids in the wheat protein was consistently higher for all the varieties grown in the Central district.   The similarity of the protein content for the samples obtained in the two districts (Table III) probably precludes the variation in the carbohydrate moiety as a factor in accounting for the differences found for the two districts.

It was thought that this difference among the samples from the two districts might be due to a greater quantity of available sulfur in the soils from the Central district.   An analysis for sulfate in the soils obtained from the locations where the wheat samples were grown revealed that the average sulfate concentration per liter of saturation extract of the soils from the Southern and Central districts amounted to 0.0075 and 0.0178 milli-equivalents, respectively.   Further studies involving the analysis for methionine in eleven samples of Comanche and Red Chief wheat grown in each of the eleven stations within the Southern and Central districts (1947 crop) revealed no significant correlation between per cent methionine and the sulfate concentration in soil samples obtained from the respective stations.   It would appear, therefore, that environmental factors other than available soil sulfate resulted in the significant differences in methionine and cystine recorded in Table III.   Differences in per cent methionine for the series of eleven samples representing Comanche and Red Chief wheat were significant for both station and variety.   Further study of the factors governing this condition merits serious attention.

The assay values for cystine and the dough mixing time for five varieties of winter wheat grown during two crop years are summarized in Table IV.   The wheat proteins contained significantly more cystine in 1947 than in 1946 and the flour from the 1947 samples also required longer mixing than did the corresponding flour samples from the 1946 crop.   Thus, there may be a relationship between mixing time and

TABLE IV

SUMMARY OF MICROBIOLOGICAL ASSAY DATA, THE ANALYSIS OF VARIANCE
OF THE PER CENT CYSTINE BASED ON TOTAL WHEAT PROTEIN,
AND FLOUR MIXING TIME FOR FIVE VARIETY COMPOSITES
OF WHEAT GROWN IN 1946 AND 1947

Crop year	Variety					Average
	Tenmarq	Comanche	E. Blackhull	Pawnee	Red Chief	
CYSTINE (PER CENT OF TOTAL WHEAT PROTEIN)[1]						
1946	2.42	2.43	2.49	2.42	2.48	2.45
1947	2.66	2.49	2.69	2.55	2.80	2.64
FLOUR MIXING TIME (MIN.)						
1946	2.3	2.8	1.4	1.2	2.2	2.0
1947	3.0	2.8	1.6	2.3	2.6	2.5

ANALYSIS OF VARIANCE FOR CYSTINE

Source of variation	Degrees of freedom	Mean squares
Variety	4	0.033
Year	1	0.273*
V×Y	4	0.016
Error	20	0.044

* Significance exceeds the 5% level.
[1] Per cent cystine in protein ($N \times 5.7$) on dry basis.   Each value is the average of three separate analyses, each performed simultaneously for all varieties grown in both years.

TABLE V

COMPARISON OF MICROBIOLOGICAL VALUES WITH THOSE CITED
IN THE LITERATURE [1]

Amino acid	Mean of microbiological values (all varieties)[2]	Literature values	
		Microbiological values	Chemical values
	%	%	%
Cystine	2.27	1.91 (1)	1.4±0.3 (4)
			2.85 (7)
Glutamic acid	33.0	35.75 (14)	
		32.4–26.4 (2)[3]	
Lysine	3.25	3.18 (19)	2.91 (4)
		3.07 (1)	
		2.74 (12)	
		2.70 (3)	
Methionine	1.43	1.32 (19)	2.19 (4)
		1.45 (1)	1.34 (11)
		2.81 (17)	2.74 (7)
		1.28 (15)	2.19 (8)
		1.08 (11)	1.26 (5)
		1.35 (3)	

[1] Expressed as per cent of the total protein ($N \times 5.7$) on dry weight basis.
[2] Data from Table III.
[3] Depending on the organism employed.

per cent cystine (based on total wheat protein) as influenced by environment.   The two samples of Comanche wheat which possessed the same optimum mixing time also contained the same percentage of cystine based on total wheat protein.   The longer mixing varieties tended to reflect greater change in mixing requirement for a small change in cystine content.   Further work relating cystine content and mixing requirement is in progress.

The reproducibility of the microbiological assay values is illustrated in Table II.   The values obtained for all four amino acids are in line with chemical and microbiological assay values for wheat reported in the literature (Table V).   The variations among reported values may in part be accounted for by sample variation.   The present work indicates that the methionine and cystine content of wheat protein may vary significantly.

Literature Cited

1. BARTON-WRIGHT, E. C. and MORAN, T.   The microbiological assay of amino acids.   II. The distribution of amino acids in the wheat grain.   The Analyst 71: 278–282 (1946).
2. BAUMGARTEN, W., MATHER, ADALINE N., and STONE, L.   Glutamic acid content of feed materials.   Cereal Chem. 22: 514–521 (1945).
3. BAUMGARTEN, W., MATHER, ADALINE N., and STONE L.   Essential amino acid composition of feed materials.   Cereal Chem. 23: 135–155 (1946).
4. BLOCK, R. J., and BOLLING, D.   The amino acid composition of proteins and foods.   Charles C. Thomas; Springfield (1945).
5. CSONKA, FRANK A.   Amino acids in staple foods.   I. Wheat (Triticum vulgare).   J. Biol. Chem. 118: 147–153 (1937).
6. DUNN, M. S., SHANKMAN, S., CAMIEN, M. N., FRANKL, W., and ROCKLAND, L. B.   Investigations of amino acids, peptides, and proteins.   XVIII. The amino acid requirements of Leuconostoc mesenteroides P-60.   J. Biol. Chem. 156: 703–713 (1944).
7. EVANS, R. J.   Determination of the cystine and methionine content of plant and animal material by a differential oxidation procedure.   Arch. Biochem. 7: 439–445 (1945).
8. GRAU, C. R., and ALMQUIST, H. J.   The methionine content of feedstuff proteins.   Arch. Biochem. 6: 287–294 (1945).
9. GREAVES, J. E., and BRACKEN, A. F.   The sulfur content of wheat.   Cereal Chem. 14: 578–581 (1937).
10. GUBLER, C. J., and GREAVES, J. E.   Cystine content of wheat and its quantitative determination.   Food Res. 7: 405–413 (1942).
11. HORN, M. J., JONES, D. B., and BLUM, A. E.   Colorimetric determination of the methionine in proteins and foods.   J. Biol. Chem. 166: 313–320 (1946).
12. HORN, M. J., JONES, D. B., and BLUM, A. E.   Microbiological determination of lysine in proteins and foods.   J. Biol. Chem. 169: 71–76 (1947).
13. KUIKEN, K. A., NORMAN, W. H., LYMAN, C. M., HALE, F., and BLOTTER, L.   The microbiological determination of amino acids.   I. Valine, leucine and isoleucine.   J. Biol. Chem. 151: 615–626 (1946).
14. LYMAN, C. M., KUIKEN, K. A., BLOTTER, L., and HALE, F.   The microbiological determination of amino acids.   II. Glutamic acid.   J. Biol. Chem. 157: 395–405 (1945).

15. LYMAN, C. M., MOSELEY, O., BUTLER, B., WOOD, S., and HALE, F.   The micro-
    biological determination of amino acids.   III. Methionine.   J. Biol. Chem.
    166: 161–171 (1946).
16. OSBORNE, T. B., and MENDEL, L. B.   The nutritive value of the wheat kernel
    and its milling products.   J. Biol. Chem. 37: 557–601 (1919).
17. RIESEN, W. H., SCHWEIGERT, B. S., and ELVEHJEM, C. A.   Microbiological
    determination of methionine in proteins and foodstuffs.   J. Biol. Chem. 165:
    347–358 (1946).
18. RIESEN, W. H., SPENGLER, H. H., ROBBLEE, A. R., HANKES, L. V., and ELVEH-
    JEM, C. A.   Cystine and related compounds in the nutrition of lactic acid
    bacteria.   J. Biol. Chem. 171: 731–748 (1947).
19. STOKES, J. L., GUNNESS, M., DWYER, I. M., and CASWELL, M. C.   Micro-
    biological methods for the determination of amino acids.   II. A uniform
    assay for the ten essential amino acids.   J. Biol. Chem. 160: 35–49 (1945).

CAKE PROPERTIES IN RELATION TO FLOUR PARTICLE SIZE FRACTIONS [1]

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ABSTRACT

Patent, straight, and clear grade flours milled from an Ohio soft red
winter wheat were fractionated into three particle size groups measured by
diameters of 37μ or less, over 37μ up to and including 53μ, and larger than
53μ.   Differences in granulation of the three flour grades were minor, but the
quantity of coarse material increased as the flour grade decreased.

The properties of the different particle size fractions from each grade
of flour varied widely.   The $0–37\mu$ fractions for each flour were lowest in
protein, ash, and viscosity and produced the best cakes.   The fractions
containing the largest sized particles were intermediate in protein and ash
content, had the highest viscosity, and produced the poorest cakes.   The
$37–53\mu$ fractions were highest in protein and ash content, and intermediate
in viscosity and cake baking quality.

Wichser and Shellenberger (6, 7, 8) have reported previously on
comparative granulation studies of flour produced from three classes
of wheat.   Various chemical and physical tests, including baking
response, were reported for the different particle size fractions.   How-
ever, only one soft wheat was tested and the cookie test was the only
baking test applied.   The present study expands the flour granulation
research by including cake baking tests of several flour fractions from
three grades of soft wheat flour.

Materials and Methods

Patent, straight, and clear grade cake flours were milled from the
same Ohio soft red winter wheat.   All samples were bleached with

TABLE I

ANALYSIS OF SOFT WHEAT FLOUR PARTICLE SIZE FRACTIONS[1]

Flour grade	Flour fraction size[2]	Protein	Ash	pH	Viscosity
	microns	%	%		°*MacM.*
Short patent	Control	7.5	0.33	5.0	28
	0–37	6.5	.32	5.0	19
	37–53	9.1	.37	5.0	78
	>53	8.7	.33	5.0	81
Straight	Control	7.6	.39	5.1	25
	0–37	6.7	.37	5.1	17
	37–53	9.2	.46	5.1	60
	>53	9.1	.40	5.1	83
Clear	Control	7.7	.49	5.2	20
	0–37	6.9	.46	5.2	13
	37–53	9.2	.57	5.2	45
	>53	9.0	.52	5.2	95

[1] Results reported on 14% moisture basis.
[2] 0–37μ inclusive, over 37μ up to and including 53μ, and over 53μ.

a commercial preparation containing benzoyl peroxide and chlorinated. The pH values are shown in Table I.

The flours were separated into three fractions based on particle size using Tyler wire screens and a Ro-Tap shaker as described by Wichser

TABLE II

CAKE BAKING FORMULA AND MIXING PROCEDURES

Ingredients	Percentage based on flour	Mixing procedure[1]
	%	
Flour	60.0	Mix for 1.5 min. at 62 rpm. Mix for 1 min. additional time at 128 rpm.
Emulsified shortening	40.0	Scrape down and continue to mix for ½ min. and again scrape down.
Water	45.0	
Sugar (sucrose)	125.0	Add all the water. Then add the
Flour	40.0	blended and sifted dry material. Mix
Salt	2.5	for 2 min. at 62 rpm. Scrape down.
Baking powder	5.5	Continue mixing at same speed for 1
Milk solids (fat-free)	15.0	additional minute.
Phosphate (V-90)	0.5	
Egg white	60.0	Mix egg whites and water, adding one-half of the mixture gradually during a period of 1 min. at 62 rpm. Scrape down. Mix an additional minute at same speed. Add balance of liquid gradually during 1 min. at 62 rpm.
Water	40.0	Scrape down. Mix 3 min. at 62 rpm., then finish mixing at 128 rpm. for ½ min.

[1] Kitchen Aid Model G mixer equipped with 4-quart bowl was used.
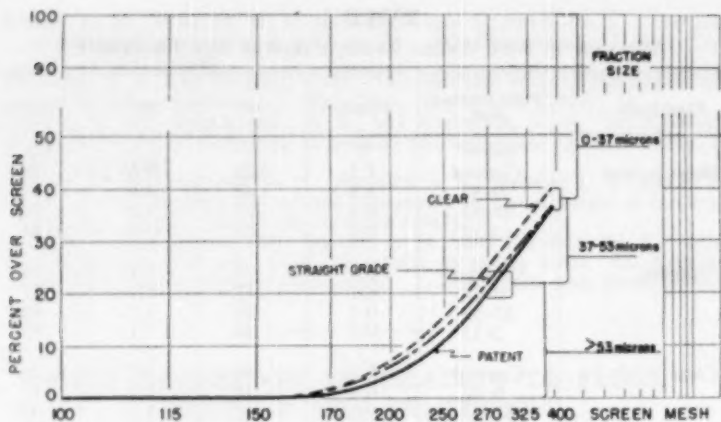
Fig. 1.   Granulation curves of patent, straight, and clear grade, soft wheat flours.
(0–37μ inclusive, over 37μ up to and including 53μ, and over 53μ.)

and Shellenberger (6, 7, 8).   The analyses of the original flours and the flour fractions are shown in Table I.

Viscosity determinations were made on acidulated flour-water
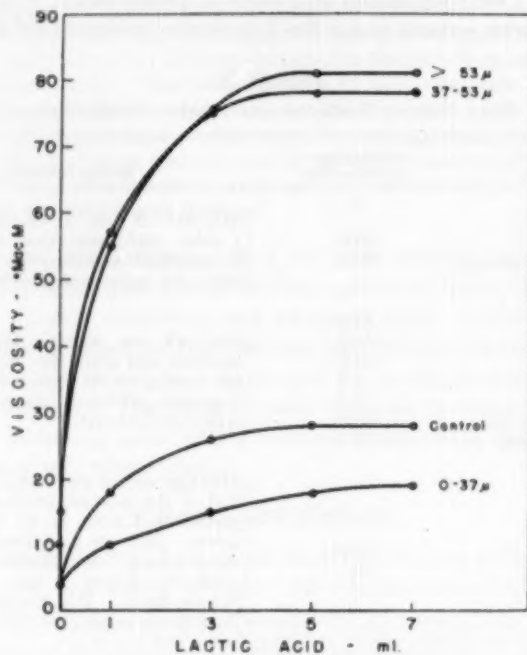
Fig. 2.   Viscosity curves of particle size fractions from patent cake flour.
(0–37μ inclusive, over 37μ up to and including 53μ, and over 53μ.)

suspensions using the MacMichael viscosimeter as described in Cereal Laboratory Methods, 5th ed. (1).

Cake baking tests were conducted using a commercial white cake formula. The ingredients and the mixing procedure are recorded in Table II. The batters (400 g.) were placed in round, 8 × 1.5 in. pans, and baked for 25 min. at 375°F.
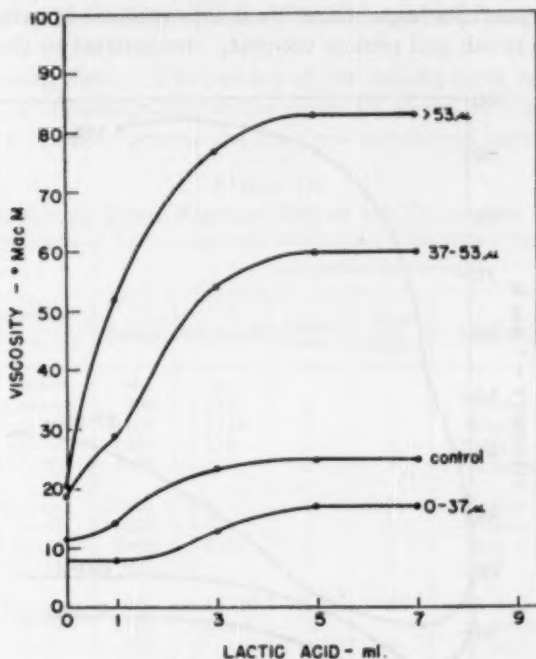
Fig. 3.   Viscosity curves of particle size fractions from straight grade cake flour. (0-37μ inclusive, over 37μ up to and including 53μ, and over 53μ.)

Results

Granulation of Cake Flours.   The granulation curves for the three flours are shown in Fig. 1, using the cumulative direct plot diagram (6). The proximity of the curves indicates that the differences in granulation were slight between the patent, straight, and clear grade flours milled from the same wheat blend.   All three flours were finely ground, compared with hard wheat flours, as is shown by the fact that less than 40% of the flour particles fail to pass through a No. 400 Tyler wire screen.   Approximately 70% of the usual hard wheat flour fails to pass through a No. 400 screen.

Although the granulation curves for the three flours are similar, the

curves show that for each particle size fraction the amount of coarser material increases as the flour grade decreases.

Ash and Protein in Cake Flour Fractions. The same relationship between particle size, ash, and protein exists as has been reported previously for soft wheat flour (6, 7). The smallest particle size group $(0-37\mu)$ is lowest in ash and protein. The intermediate particle size fraction $(37-53\mu)$ is highest in both ash and protein. The flour fraction containing particles larger than 53μ is intermediate between the other two groups in ash and protein content. In contrast to the results ob-
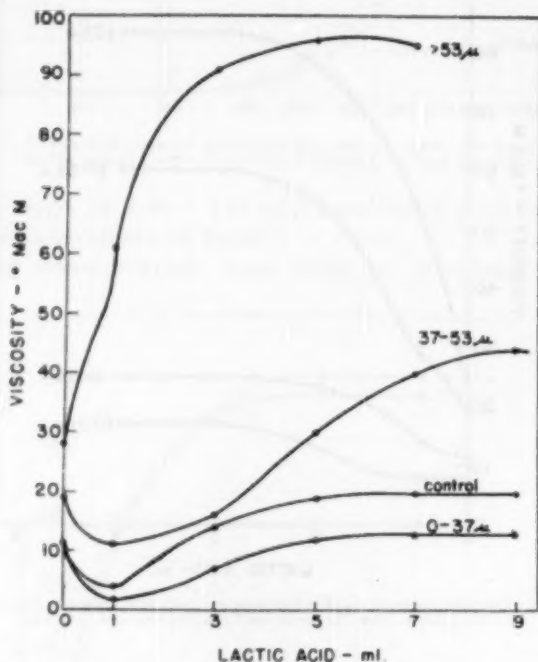
Fig. 4. Viscosity curves of particle size fractions from clear grade cake flour. (0–37μ inclusive, over 37μ up to and including 53μ, and over 53μ.)

tained with hard wheat flour (8) in which the small particle size fraction $(0-37\mu)$ was low in protein but high in ash, the soft wheat flour fractions show a direct relationship throughout between ash and protein. Fractions highest in protein were found to be highest in ash.

Viscosity of Cake Flour Fractions. The relationship between Mac-Michael viscosity and flour particle size for the three flour types is illustrated graphically in Figs. 2, 3, and 4. Viscosity increased greatly with increase in flour particle size. In every case the smaller particle size fraction $(0-37\mu)$ had the lowest viscosity while the largest particle

size fraction had the highest viscosity. Since approximately 60% of the flour consisted of the 0-37μ fraction, it would be expected that the viscosity of the original flour (control) would be similar to the curve for the smallest size particle group. The expected greater buffering effect (3) of higher ash flour is apparent in the clear flour measurements.

There was an apparent relationship between viscosity readings and the quality of the cakes produced from the different flour fractions. With one exception the low viscosity flour fractions produced the best cakes.

Cake Baking Tests. The results of the baking tests are recorded in Table III and photographs of the cakes are shown in Figs. 5, 6, and 7. For all three flour grades the fractions containing particles within

TABLE III

THE EFFECT OF FLOUR PARTICLE SIZE ON THE QUALITY OF CAKES

Flour grade	Flour fractions[1]	Cake grading characteristics					
		Volume	Vol. index[2]	Appear-ance	Grain and texture	Crumb color	Rank
	microns	*ml.*	*in.*				
Patent	Control	980	2 7/8	4	4	Good	4
	0-37	1100	3 5/16	1	1	Bright	1
	37-53	1050	3 1/8	2	2	Gray	2
	>53	1000	3	3	3	Sl. gray	3
Straight	Control	1005	3	2	2	Bright	2
	0-37	1070	3 1/4	1	1	Bright	1
	37-53	1040	3 1/8	3	3	Dull	3
	>53	983	3	4	4	Dull	4
Clear	Control	985	2 11/16	4	2	Sl. dull	2
	0-37	1108	3 1/4 '	1	1	Sl. bright	1
	37-53	1030	3	2	3	Sl. gray	3
	>53	1013	2 13/16	3	4	Gray	4

[1] 0-37μ inclusive, over 37μ up to and including 53μ, and over 53μ.
[2] Volume index obtained by slicing layer in half and placing the two halves one on top of the other. The measurement is the thickness at the center of the two half slices of cake. A top quality cake should measure 3 1/4 in. in height.

the size range of 0-37μ produced the best cakes and were ranked No. 1. It is evident in these studies that cake quality improved with a decrease in particle size. This corroborates the work of Schreck and Gerrits (5) and Alexander (2). The only exception to this observation was the patent grade for which the unfractionated flour produced less satisfactory cakes than did any of the fractions from the same sample. Since all particle size groups of the short patent flour produce good cakes, uniformity of particle size may be more important than particle size. Kress (3) has stated that for bread baking purposes the more uniform the granulation the better the baking quality, and Patterson

Fig. 5.   Cakes made from patent grade flour fractions.   (0–37μ inclusive, over 37μ up to and including 53μ, and over 53μ.)

Fig. 6.   Cakes made from straight grade flour fractions.   (0–37μ inclusive, over 37μ up to and including 53μ, and over 53μ.)
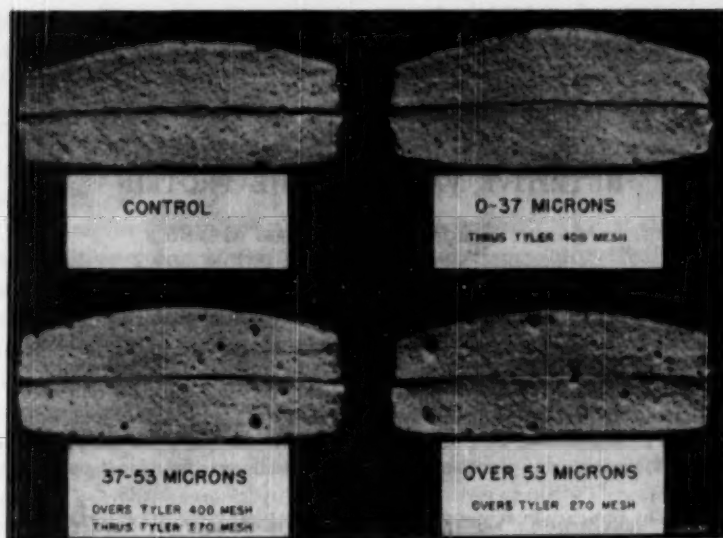
Fig. 7.   Cakes made from clear grade flour fractions.   (0–37μ inclusive,
over 37μ up to and including 53μ, and over 53μ.)

(4) has indicated that the cake-making qualities of a flour were dependent on uniformly fine division of the flour particles.

The studies dealing with three grades of soft red winter wheat flour showed that the cake baking properties of the flour fractions improved with a decrease in particle size and a decrease in viscosity.

Acknowledgments

Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS.   Cereal Laboratory Methods. 5th ed.   Amer. Assoc. Cereal Chemists: St. Paul, Minn. (1947).
2. ALEXANDER, GEORGE L.   Soft-wheat testing problems.   Cereal Chem. 16: 197–208 (1939).
3. KRESS, C. B.   Granulation of flour and its relation to baking quality.   Cereal Chem. 6: 202–214 (1929).
4. PATTERSON, P. M.   The cake flour laboratory.   Cereal Chem. 1: 159–161 (1924).
5. SCHRECK, A. G., and GERRITS, M. J.   Evaluation and selection of cake flours. Res. Bul. No. 35, Series AA, Siebel Institute of Technology, Chicago (1930).
6. WICHSER, FRANK W., and SHELLENBERGER, J. A.   Methods for determining flour particle size distribution.   Cereal Chem. 25: 155–167 (1948).
7. WICHSER, FRANK W., and SHELLENBERGER, J. A.   Comparative granulation studies of flour produced from three classes of wheat.   Assoc. Operative Millers Bull. pp. 1682–1686, July (1948).
8. WICHSER, FRANK W., and SHELLENBERGER, J. A.   Comparative studies in granulation of hard red winter, hard spring and soft wheat flours.   Mill. Prod. Vol. 13, No. 7, pp. 1, 7, 27–29 (1948).

THE *IN VITRO* DIGESTIBILITY OF RAW AND HEAT-PROCESSED SOY PRODUCTS VARYING IN THE NUTRITIVE VALUE OF THE PROTEIN [1]

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ABSTRACT

The susceptibility of the protein in commercially available soy products to *in vitro* enzymic digestion has been compared with the nutritional value obtained by animal assay methods, using soy products in various stages of heat-processing. These consisted of: (1) dehulled soybeans, (2) unheated, defatted soy flakes, (3) mildly heat-processed soy flour, (4) soy flour, optimally heat-processed with respect to the nutritional quality of the protein, and (5) an over-heat-processed flour experimentally produced for the present study.

The presence in raw soy products of a naturally-occurring proteolytic factor interfered with the *in vitro* digestion technique by acting synergistically with the pancreatin employed in the test. However, poor correlation was obtained between the nutritive data and the *in vitro* digestibility values for the protein in the soy flour samples, even when the interfering factor was inactivated. Large increases in the efficiency with which the protein in the soy products was utilized by the test animals were associated with only small increases in *in vitro* digestibility. However, in the case of the sample which had been purposely overheated, a large increase in the susceptibility of the protein to *in vitro* digestibility has been noted, and this is associated with only a small decline from the optimum nutritive value.

The nutritive value of the protein in soy products can be markedly increased without appreciably affecting antitryptic activity. The findings supplement but do not contradict earlier reports in the literature pointing to an inverse correlation between the protein value of soy products and antitryptic potency.

The improvement in nutritive value of soybean protein, effected by heat treatment in the presence of water, has been demonstrated by numerous investigators in laboratory feeding experiments with chicks (18), mice (33), and rats (20). Various suggestions have been offered to explain this increase in the biological value of the protein. A substance capable of inhibiting the activity of trypsin was found in raw soybean oil meal by Ham and Sandstedt (15) and by Bowman (8). This substance was later crystallized by Kunitz (22) and shown to be a

protein.   The naturally-occurring trypsin inhibitor has been held to be largely responsible for the interference in the biological utilization of the protein.   Addition of the inhibitor to otherwise adequate diets was shown to impair the growth rate of chicks (16) and of rats (20). That the inhibitor is destroyed or rendered inactive by heat has been demonstrated in both *in vivo* (20, 32, 33) and *in vitro* (7, 33) experiments.

It was emphasized by Melnick and associates (27) that the increase in nutritive value of the protein resulting from heat treatment was accompanied by an increase in the susceptibility of the protein to *in vitro* pancreatic digestion.   In support of their findings, Evans (12, 14), using trypsin or trypsin and erepsin, Jones (19), using trypsin, and Riesen *et al.* (30), using pancreatin, observed that a marked increase in *in vitro* digestibility occurred when soybean oil meal was autoclaved or cooked in water.   Harte (17), using egg white, in which the existence of a trypsin inhibitor has been conclusively demonstrated (2, 24), found that the heat-coagulated material was rapidly digested *in vitro* by pancreatin while the unheated material showed no significant digestion even after 24 hours.   In casein, however, where heating was known to impair the nutritive value of the protein, its susceptibility to *in vitro* enzymic digestion was found to be concomitantly decreased (4, 29).

The present investigation is part of an over-all study, undertaken to characterize soy products used as such or as ingredients in food formulations for human feeding.   The paper (25) preceding this one is concerned with an evaluation of the functional (utility) properties of the protein in commercially available soy products, with special emphasis on curd-producing capacity.   The current study was designed to determine the relationship between the nutritive value and *in vitro* digestibility of the protein, and to determine therefrom the suitability of the *in vitro* digestion technique as an objective test procedure for characterizing soy products with regard to nutritive properties.

Materials and Methods

The soybean flours employed in this study were all prepared from the same batch of soybeans and were representative of raw and heat-treated commercial products.   These samples were furnished by the Soya Food Research Council, Soy Flour Association.   The products consisted of: (1) dehulled soybeans, (2) unheated, solvent-extracted soy flakes, (3) bakery-type soy flour, mildly heated in order to eliminate the bitter qualities of the raw bean but not sufficiently heated to seriously impair its functional properties, (4) a product optimally heat-processed with respect to the nutritive value of the protein, and (5)

an overheated product experimentally produced for the present study. Samples 2, 3, 4, and 5, commercially solvent-extracted, were ground and sifted through a No. 100 U. S. Standard sieve. Sample 1, the dehulled soybeans, was first ground through a sieve with openings 1 mm. in diameter, defatted with Skellysolve B, and finally ground to pass a No. 100 sieve.

Special equipment was employed in the commercial preparation of sample 2, in order to keep at a minimum the heat involved in solvent extraction of the oil and subsequent removal of the solvent from the product. In the case of sample 3, the bakery-type soy flour, the amount

TABLE I

INFLUENCE OF HEAT-PROCESSING ON THE PROPERTIES OF COMMERCIALLY PRODUCED SOYBEAN PRODUCTS [1]

Sample/number and description	1. Unextracted dehulled soybeans	2. Solvent extracted, unheated	3. Solvent extracted, bakery type	4. Solvent extracted, optimally heated	5. Solvent extracted, overheated
Moisture, %	6.1	7.3	7.2	6.4	4.2
Fat, %	21.3	0.9	0.6	0.6	0.4
Total protein, %	41.9	52.1	53.6	54.1	55.5
Water soluble protein, % of total	76.7	83.1	65.5	22.5	6.2
β-amylase[2] (°Lintner)	110.0	135.0	5.4	0.9	2.7
Urease activity[3] (increase in pH)	1.75	1.90	1.70	0.2	0.05
Protein efficiency (g. gained/g. protein consumed)	0.65	0.79	1.52	1.96	1.78

[1] Samples and data were supplied by Dr. J. W. Hayward, Archer-Daniels-Midland Co. Minneapolis, Minnesota, acting in behalf of the Soya Food Research Council, Soy Flour Association. All samples were from the same batch of soybeans.
[2] Modified procedure of the A.O.A.C., 6th ed. (1945). The β-amylase activity is determined by its hydrolytic action on starch. The maltose produced is measured by the reduction of a standard solution of potassium ferricyanide.
[3] Procedure of Caskey, C. D., and Knapp, F. C., Ind. Eng. Chem., Anal. Ed. 16: 640 (1944). The increase in pH is due to the liberation of ammonia from urea by the action of urease.

of heat used was the least necessary, within the limitations of conventional equipment, to eliminate the bitter qualities of the material.

The data presented in Table I are indicative of the degree of heat treatment to which the samples were exposed during commercial processing. The insignificance of the heat involved in the preparation of sample 2, the raw product, is evidenced by the retention of all its heat-labile properties. The mild heat treatment accorded sample 3, the bakery-type product, is manifested by its having suffered only a slight loss in urease activity and in water solubility of the protein. Caskey and Knapp (9) and Bird et al. (3) concluded from the results of their studies of the effect of heat on the urease activity and nutritive

value of the soybean protein that an increase in pH of 1.0 or more in the urease test employed by them indicated that the soybean meal had been inadequately heated to attain optimum nutritive value of the protein. β-amylase, on the other hand, because of its extreme heat-lability, is rapidly inactivated even at relatively low temperatures (10), and cannot adequately be employed to distinguish products that have been heated mildly from those which have been more severely heated. It will be observed that the protein solubility of the optimally heated product has been considerably reduced, while that of the excessively heated product has been almost entirely eliminated. The urease and β-amylase activity of both products are negligible.

In Vitro Digestibility. The susceptibility of the protein to *in vitro* enzymic digestion was determined according to the procedure of Melnick and associates (27, 28). A sample containing 6 gm. of protein ($N \times 6.25$) was suspended in 50–60 ml. of water and the pH adjusted to 8.4. Thirty ml. of 0.075 M phosphate buffer solution [a] at the same pH were then added to the suspension and the volume brought to 130 ml. with water. Following the addition of 20 ml. of the buffer solution containing 200 mg. of U.S.P. pancreatin,[b] the suspension was thoroughly mixed, covered with 10 ml. of toluene, and incubated at 37°C. Five ml. aliquots of the enzymic digests were withdrawn at fixed intervals and the degree of hydrolysis determined by the increase in formol titratable nitrogen. The titration values for the undigested materials were determined on aliquots of similarly prepared samples to which heat-inactivated pancreatin had been added. The maximum formol titration values were obtained on acid hydrolysates of the samples.[c] The degree of hydrolysis at a given interval was expressed as the increase in the formol titratable nitrogen at that interval, divided by the maximum increase in this value (i.e., the figure for the acid hydrolyzed sample). The value thus obtained multiplied by 100 gave the per cent hydrolysis.

Antitryptic Activity. The inhibitor potency of the soy products was determined by a modification of the procedure described by West-fall and Hauge (33). This method involves the measurement of the ability of the anti-tryptic factor contained in the soy flour to retard the *in vitro* pancreatic digestion of casein. Twenty ml. of 0.5% pancreatin suspension buffered at pH 8.3 were pipetted into a glass-stoppered Erlenmeyer flask which was then placed in a 37°C. water bath. Five ml. of 0.5% buffered flour suspension were then added to the pancreatin, followed in exactly 5 minutes by 25 ml. of 4% sodium

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[a] 750 ml. of $M/5$ KH₂PO₄ plus 144 ml. of 1 M NaOH diluted to 1,000 ml.: pH 8.4.
[b] Purchased from Central Scientific Co., Chicago, Ill.
[c] A sample containing 6 gm. of protein was refluxed for 24 hours with 8 N H₂SO₄. The hydrolysate was cooled, neutralized, and diluted to 130 ml. Aliquots were withdrawn, buffer added to the same concentration as in aliquots of the enzymic digests, and assayed by formol titration.

caseinate solution similarly buffered.   Aliquots were withdrawn at
specific intervals and the liberated amino nitrogen was measured by
the formol titration technique.   Corrections were made for the initial
formol titratable nitrogen and that contributed by the inhibitor
carrier, in this case the soy flour.   The increase in formol titration was
plotted against time of digestion and the relative velocity constant
calculated from the reciprocal of the time required to decompose a
fixed quantity of substrate.   Inhibition was expressed as the per cent
decrease from the relative velocity constant obtained when casein
digestion was unretarded.   Details of the procedure will be published
at a later date.

   Protein Efficiency.   The nutritional data presented here were
supplied by Mr. Ralph Holder of the Central Soya Company, Decatur,
Indiana.   Diets containing the soy products as the source of protein
were fed *ad libitum* to duplicate groups of five weanling male albino
rats of the Sprague-Dawley strain for a period of eight weeks during
which food consumption and body weight changes were recorded at
regular intervals.   The ration furnished approximately 11% protein
($N \times 6.25$).   Protein efficiency was expressed as the grams of weight
gained per gram of protein consumed.   In addition to the protein
contributing component, the composition of the basal diet was as
follows: 10% sucrose; 3% ruffex; 4% salt mixture (Wesson Modifica-
tion of the Osborne-Mendel salt mixture) (31); 2% 400-D/2,000-A oil,
with 1.25 mg. α-tocopherol added per g. of oil; 1% condensed fish
solubles; 2% vitamin mixture; an amount of soybean oil sufficient
to make 10% when added to the oil present in the test sample; corn
starch to make a total of 100%.   The vitamin mixture supplied per
100 g. of feed: 400 μg. thiamine; 400 μg. riboflavin; 400 μg. pyridoxine;
3.5 mg. niacin; 1.1 mg. calcium pantothenate; 7.5 mg. para-amino
benzoic acid; 30 mg. inositol; 200 mg. choline chloride; and 400 μg.
menadione.   1% of condensed fish solubles (50% solids) were used
to supply the unidentified growth factors.

Results and Discussion

   The results obtained in the digestibility and anti-tryptic activity
tests are presented in Table II, together with the nutritional values
(efficiency of protein utilization) for the five samples.   It will be noted
that the mild heat treatment employed in processing the bakery-type
flour has produced no measurable loss in its *in vitro* anti-tryptic
activity.   The nutritional value, on the other hand, has been consider-
ably improved.   In the optimally heated sample, the large increase
in protein efficiency was accompanied by a marked decrease in anti-
tryptic activity.   However, in the case of the so-called "overheated"

sample, where the anti-tryptic activity has been still further reduced, efficiency of protein utilization has not been improved; actually, a slight decline from the optimum resulted. The impairment in biological value of the protein due to excessive heating has been attributed to a loss in the biological availability of lysine (5, 11, 26, 29) and methionine (13, 21). In their studies of the heat destruction of the soybean inhibitor as related to the heat destruction of urease, Borchers *et al.* (7) observed that the inhibitor survived longer periods of heating, at atmospheric and elevated pressures, than did urease. They re-

TABLE II

THE *IN VITRO* DIGESTIBILITY, ANTI-PROTEOLYTIC ACTIVITY, AND NUTRITIVE
VALUE OF COMMERCIALLY AVAILABLE SOYBEAN FLOURS
IN VARIOUS STAGES OF HEAT-PROCESSING

Sample	Extent of hydrolysis[1]			Anti-proteolytic activity[2] (per cent inhibition)	Protein efficiency[3]
	After 1 day	After 2 days	After 5 days		
	%	%	%		gms. gained/ gms. consumed
1 Raw flour	12	16	22	57	0.65
2 Raw flour	12	15	20	57	0.79
3 Bakery-type flour	6	9	15	57	1.52
4 Optimally heated flour	13	19	23	33	1.96
5 Overheated flour	16	20	29	15	1.78

[1] Based upon the ability of the proteolytic enzymes in the test system, containing a suboptima quantity of added pancreatin, to digest the soybean protein.
[2] Based upon the ability of the soybean inhibitor to retard the *in vitro* pancreatic digestion of casein.
[3] Eight weeks' rat growth assays conducted by Mr. Ralph Holder of the Central Soya Co., Decatur, Indiana; the ration furnished approximately 11% protein ($N \times 6.25$).

ported that the urease test could not be employed as a reliable index for the adequacy of heat treatment for the destruction of the inhibitor.

Poor correlation has been obtained between the protein efficiency data and the *in vitro* digestibility values for the protein in the soy flour samples. Large increases in the efficiency with which the soy products are used by the test animals are not associated with an increase but with an apparent loss of susceptibility to *in vitro* enzymic digestion in the case of sample 3, the mildly heat treated product, and with only a slight improvement in the digestibility of sample 4, the optimally heated product. However, in the case of sample 5, which had been purposely overheated, a large increase over the value obtained for the

raw material has been effected in the susceptibility of the protein to *in vitro* enzymic digestion, and this is associated with only a small decline from the optimum nutritive value. The present findings are not considered to be inconsistent with reports in the literature where large differences between the biological value of the protein in raw soy products and that in the same product heated to presumably optimum value have shown good correlation with the results yielded by *in vitro* digestibility studies. In very few, if any, of the studies reported in the literature have both animal assays and *in vitro* digestibility tests been conducted on the same sample in various stages of heat-processing. In most cases, comparisons were made only between the raw product and the same material heated to what was presumed to be optimum nutritive value. In the present study, the protein in the excessively heated product has been found to exhibit considerable improvement (more than 100%) in biological value over that of the raw product, and also a large increase in susceptibility to *in vitro* enzymic digestion.

The impairment in the susceptibility of the soy protein to *in vitro* enzymic digestion resulting from mild heat treatment is explained by the existence in raw soy products of naturally-occurring proteolytic enzymes. The occurrence of such proteases was reported by Melnick and Oser (28) who pointed out that the proteolysis due to these enzymes and that due to the added pancreatin was synergistic rather than additive, each factor augmenting the action of the other so that the net effect is greater than the sum of the separate effects. The presence of proteases in raw soy products is indicated by the data in Table III. Incubation of the raw product without pancreatin resulted in an increase in formol-titratable nitrogen. This increase was reduced to a trace when an aqueous suspension of the material was heated for 30 minutes at 60°C. prior to incubation without pancreatin. At 80°C. no measurable increase was observed. Concurrent with the loss in activity of the soybean protease, there occurred a decrease in the *in vitro* digestibility of the protein by pancreatin, the extent of protein digestion being less than that calculated by subtracting the results obtained in the test system containing only active soy proteases from that containing the added pancreatin. The absence of any increase in formol-titratable nitrogen in the bakery-type product, when incubated without pancreatin, indicates that the naturally-occurring proteolytic factor had been inactivated by the heat-processing employed in its manufacture commercially. Consequently, no impairment in its *in vitro* digestibility by pancreatin was effected by heating its aqueous suspension at 60°C. as in the case of the raw product. However, heating the aqueous suspension at temperatures exceeding 60°C. resulted in improvement of the *in vitro* digestibility. This

improvement may be attributed to the effect of the additional heat treatment in increasing the denaturation of the protein or upon the destruction of the soybean inhibitor or to a combination of both. The greater *in vitro* digestibility of the protein or the raw product when heated (in aqueous suspension) at 80°C. and 95°C. than that of the same product heated at 60°C. may be explained in the same manner. The higher temperatures were capable of inactivating the soybean

TABLE III

INFLUENCE OF NATURALLY-OCCURRING PROTEOLYTIC ENZYMES
ON THE *IN VITRO* DIGESTIBILITY OF SOYBEAN PROTEIN

Sample	Experiment		Extent of hydrolysis	
	No.	Description	After 1 day	After 5 days
			%	%
Raw soy flour	1	Incubated with pancreatin	10	18
	2	Incubated without pancreatin	1	3
	3	Heated in aqueous suspension for 30 minutes at 60°C., then incubated with pancreatin	6	13
	4	Same as 3, but without pancreatin	0	1
	5	Heated in aqueous suspension for 30 minutes at 80°C., then incubated with pancreatin	8	16
	6	Same as 5, but without pancreatin	0	0
	7	Heated in aqueous suspension for 30 minutes at 95°C., then incubated with pancreatin	10	20
	8	Same as 7, but without pancreatin	0	0
Bakery-type soy flour	9	Incubated with pancreatin	5	10
	10	Incubated without pancreatin	0	0
	11	Same as 3	6	10
	12	Same as 3, but without pancreatin	0	0
	13	Same as 5	8	16
	14	Same as 5, but without pancreatin	0	0
	15	Same as 7	10	20
	16	Same as 7, but without pancreatin	0	0

proteases in the test system, but this effect, contributing to decreased digestibility, was masked by the concomitant denaturation of the soy proteins rendering them more susceptible to *in vitro* pancreatic digestion.

It was apparent that, in order for the *in vitro* digestibility test to be applied to the characterization of soy products with respect to the nutritional value of the protein component, it would be necessary to

render inactive any proteolytic enzymes that might be naturally present. To accomplish this, the soy flour samples were subjected to a standardized heat treatment in the laboratory prior to being assayed for *in vitro* digestibility. Samples containing 6 g. of protein were suspended in 60 ml. of water and the suspensions heated in a boiling water bath for 15, 30, and 60 minutes. Susceptibility of the protein to *in vitro* enzymic digestion was determined in the previously described manner. The higher temperature was selected to assure

TABLE IV

INFLUENCE OF STANDARDIZED LABORATORY HEAT TREATMENT * ON THE *IN VITRO* DIGESTIBILITY OF COMMERCIALLY AVAILABLE SOYBEAN FLOURS IN VARIOUS STAGES OF HEAT-PROCESSING

Sample No.	Standardized laboratory heat treatment	Extent of hydrolysis		
		After 1 day	After 2 days	After 5 days
	minutes	%	%	%
1	None	12	16	22
Raw flour	30	12	15	23
2	None	12	15	20
Raw flour	15	12	15	20
	30	13	15	20
	60	13	16	21
3	None	6	9	15
Bakery-type flour	15	10	13	20
	30	13	17	22
	60	15	18	23
5	None	13	19	23
Optimally heat-processed flour	15	14	18	24
	30	15	18	24
	60	15	20	24
5	None	16	20	29
Overheated flour	15	21	25	29
	30	22	25	28
	60	22	26	29

* Aqueous suspension of 1 part flour in 6 parts of water heated in a boiling water bath for the indicated length of time. The temperature of the suspension was approximately 95°C.

complete inactivation of the naturally-occurring enzymes, since the retention of only a small amount could result in relatively large increases in *in vitro* digestibility due to synergism with pancreatin.

Shown in Table IV are the results of these experiments. Although the *in vitro* digestibility of the raw product did not appear to be significantly improved by this laboratory heat treatment, it should be recognized that the digestibility prior to the heat treatment was in part due to the synergism between the naturally-occurring proteolytic enzymes and the added pancreatin. The change in *in vitro* digestibility

due to the standardized laboratory heat treatment should more properly be measured from the otherwise lower value that would have been obtained in the absence of naturally-occurring proteolytic activity as indicated in Table III. Taking this into account, the increase in *in vitro* digestibility, though not apparent here, would indeed be large.
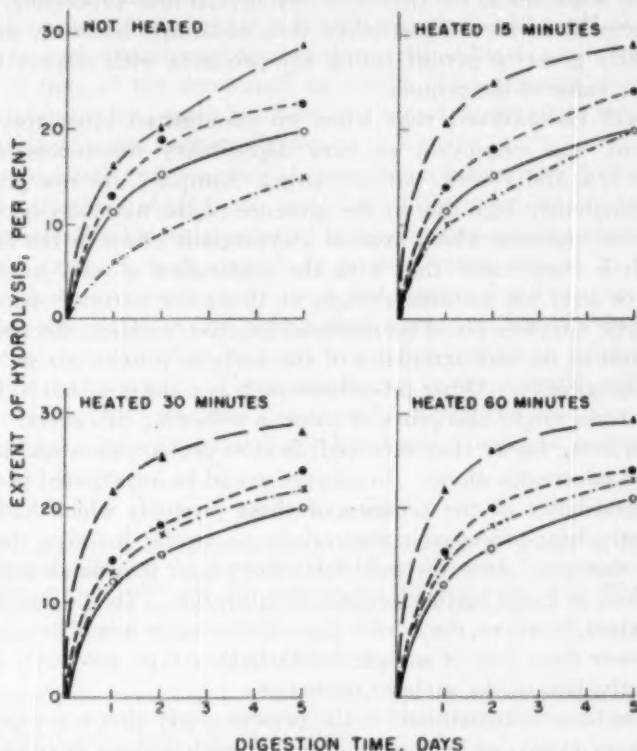
Fig. 1. Influence of standardized laboratory heat treatment on the *in vitro* digestibility of commercially heat-processed soybean products.

O—Raw soy flour (Sample No. 2)
X—Mildly heated bakery-type flour (Sample No. 3)
●—Heated to optimal protein efficiency (Sample No. 4)
▲—Overheated with respect to protein efficiency (Sample No. 5)

The bakery-type product, whose naturally-occurring proteolytic activity had been inactivated by commercial heat processing, exhibited considerable improvement as a result of the laboratory heat treatment. After having been heated for 30 minutes, its digestibility value even exceeded, although to a small degree, that of the similarly heated raw product. In the case of the optimally heat-processed product only a slight improvement was observed; while in the over-

heated sample the improvement occurred in the first 15 minutes of the laboratory heat treatment, with no further change upon extending the heating time to 60 minutes. It will be noted in Fig. 1 that when the soy flour suspensions in water were subjected to the standardized laboratory heat treatment (95°C.) for periods of 30 and 60 minutes prior to being assayed, the *in vitro* digestibility values obtained were in the same sequence as the degree of commercial heat-processing. The differences in *in vitro* digestibility thus obtained, however, are not sufficiently great to permit rating soy products with respect to the nutritive value of the protein.

It will be observed that when no standarized laboratory heat treatment was employed, *in vitro* digestibility differences among samples 3, 4, and 5 were relatively large. Sample 2, the raw product, was anomalously high due to the presence of the naturally-occurring proteolytic enzymes which exerted a synergistic effect in the *in vitro* test. It is conceivable that with the application of very mild heat (60°C. or less) for suitable periods of time, the naturally-occurring proteolytic enzymes could be rendered inactive without effecting any alterations in the characteristics of the soybean protein *per se*, which affect digestibility. Other procedures such as using dry heat at higher temperatures might also prove of value in achieving this effect. With the interfering factor thus removed, *in vitro* proteolysis would be due to added pancreatin alone. No change would be anticipated in the *in vitro* digestibility of the proteins of those products which had been sufficiently heat-processed commercially to render inactive the proteolytic enzymes, since the mild laboratory heat treatment would be insufficient to cause further protein denaturation. In the case of the raw product, however, the *in vitro* digestibility value would be expected to be lower than that of sample 3 (the bakery-type product), due to the inactivation of the soybean proteases.

It has been demonstrated in the present study that a soy product (the bakery-type) can be heat-processed in such manner as to improve appreciably the nutritional value of the protein without impairment of its anti-tryptic activity *in vitro*. In this respect, the product resembles spray-dried egg albumen, which exhibits both high nutritional value and high anti-tryptic activity *in vitro* (1). Borchers *et al*. (6) have demonstrated that with increasing laboratory manipulation, it was possible to obtain a highly concentrated soybean trypsin inhibitor (by *in vitro* measurement), with insignificant growth-depressing properties. It must not be overlooked that each of the steps involved in the extended laboratory process permits the occurrence of some protein denaturation. Under such conditions of incipient protein denaturation, it is possible that certain properties of the anti-

tryptic complex (viz., those responsible for growth inhibition) may be altered while others remain unchanged (viz., anti-trypsin activity *in vitro*). Another likely explanation may be that the inhibitor complex, in its condition of incipient denaturation, becomes susceptible to digestion by pepsin so that its anti-tryptic activity *in vivo* is no longer apparent. It has been demonstrated by Kunitz (23) that the soy inhibitor in its native state is scarcely affected by pepsin at pH 3.0. When denatured, however, it is readily digested. At a pH of 2.0 or lower, the native inhibitor becomes slowly digestible at a rate less than 1/500 of that of the denatured material. This digestibility of the native inhibitor by pepsin has been attributed to the slight denaturation effected by the acid conditions.

The possibility of coupling the results of the *in vitro* digestibility studies with those obtained in our laboratories in evaluating the soy-curd potentialities of soy products (25) also merits consideration. The ideal soy product, with respect to protein value, should be readily susceptible to *in vitro* digestion in a test system free from naturally-occurring soy proteases and should yield a soy curd low in volume and capable of being further reduced when the sample is subjected to further heat treatment. These approaches to the over-all problem of characterizing soy products for specific functions are now under investigation at the Quartermaster Food and Container Institute for the Armed Forces.

Acknowledgments

Literature Cited

1. ALLISON, J. B., ANDERSON, J. A., and WHITE, J. I. Evaluation of the nutritional value of proteins with normal and protein-deficient dogs. Trans. Amer. Assoc. Cereal Chem. VII: 24–33 (1949).
2. BALLS, A. K., and SWENSON, T. L. The antitrypsin of egg white. J. Biol. Chem. 106: 409–419 (1934).
3. BIRD, H. R., BOUCHER, R. V., CASKEY, C. D., JR., HAYWARD, J. W., and HUNTER, J. E. Urease activity and other chemical criteria as indicators of inadequate heating of soybean oil meal. J. Assoc. Offic. Agr. Chemists 30: 354–364 (1947).
4. BLOCK, R. J., JONES, D. B., and GERSDORFF, C. E. F. The effect of dry heat and dilute alkali on the lysine content of casein. J. Biol. Chem. 105: 667–668 (1934).
5. BLOCK, R. J., and MITCHELL, H. H. The correlation of the amino-acid composition of proteins with their nutritive value. Nutr. Abstr. and Rev. 16: 249–278 (1946–1947).
6. BORCHERS, R., ACKERSON, C. W., MUSSEHL, F. E., and MOEHL, A. Trypsin inhibitor. VIII. Growth inhibiting properties of a soybean trypsin inhibitor. Arch. Biochem. 19: 317–322 (1948).
7. BORCHERS, R., ACKERSON, C. W., and SANDSTEDT, R. M. Trypsin inhibitor. III. Determination and heat destruction of the trypsin inhibitor of soybeans. Arch. Biochem. 12: 367–374 (1947).

8. BOWMAN, D. E.   Fractions derived from soybeans and navy beans which retard tryptic digestion of casein.   Proc. Soc. Exp. Biol. Med. 57: 139–149 (1944).
9. CASKEY, C. D., and KNAPP, F. C.   Method for detecting inadequately heated soybean oil meal.   Ind. Eng. Chem., Anal. Ed. 16: 640–641 (1944).
10. CLAUS, W. D.   Kinetics of β-amylase action in 20% starch pastes at elevated temperatures.   Cereal Chem. 24: 59–69 (1947).
11. ELDRED, N. R., and RODNEY, G.   The effect of proteolytic enzymes on raw and heated casein.   J. Biol. Chem. 162: 261–265 (1946).
12. EVANS, R. J.   Hydrolysis of soybean oil meal proteins by some proteolytic enzymes.   Arch. Biochem. 11: 15–21 (1946).
13. EVANS, R. J., and McGINNIS, J.   Cystine and methionine metabolism by chicks receiving raw or autoclaved soybean oil meal.   J. Nutrition 35: 477–488 (1947).
14. EVANS, R. J., McGINNIS, J., and ST. JOHN, J. L.   The influence of autoclaving soybean oil meal on the digestibility of the proteins.   J. Nutrition 33: 661–672 (1947).
15. HAM, W. E., and SANDSTEDT, R. M.   A proteolytic inhibiting substance in the extract from unheated soybean meal.   J. Biol. Chem. 154: 505–506 (1944).
16. HAM, W. E., SANDSTEDT, R. M., and MUSSEHL, F. E.   The proteolytic inhibiting substance in the extract from unheated soybean meal and its effect upon growth in chicks.   J. Biol. Chem. 161: 635–642 (1945).
17. HARTE, R. A.   On the in vitro proteolysis of egg white.   Science 102: 563–564 (1945).
18. HAYWARD, J. W., STEENBOCK, H., and BOHSTEDT, G.   The effect of heat as used in the extraction of soybean oil upon the nutritive value of the protein of soybean oil meal.   J. Nutrition 11: 219–234 (1936).
19. JONES, D. B.   Nutritive value of soybean and peanut proteins.   Federation Proc. 3: 116–120 (1944).
20. KLOSE, A. A., HILL, B., and FEVOLD, H. L.   Presence of a growth inhibiting substance in raw soybeans.   Proc. Soc. Exp. Biol. Med. 62: 10–12 (1946).
21. KLOSE, A. A., HILL, B., and FEVOLD, H. L.   Food value of soybean protein as related to processing.   Food Tech. 2: 201–206 (1948).
22. KUNITZ, M.   Crystalline soybean trypsin inhibitor.   J. Gen. Physiol. 29: 149–154 (1946).
23. KUNITZ, M.   Crystalline soybean inhibitor. II. General properties.   J. Gen. Physiol. 30: 291–310 (1947).
24. LINEWEAVER, H., and MURRAY, C. W.   Identification of the trypsin inhibitor of egg white with ovomucoid.   J. Biol. Chem. 171: 565–581 (1947).
25. LOSKA, S. J., JR., and MELNICK, D.   Laboratory procedure for evaluating the curd-producing capacity of soy products.   Cereal Chem. 27: 127–140 (1950).
26. McGINNIS, J., and EVANS, R. J.   Amino acid deficiencies of raw and overheated soybean oil meal for chicks.   J. Nutrition 34: 725–732 (1942).
27. MELNICK, D., OSER, B. L., and WEISS, S.   Rate of enzymic digestion of proteins as a factor in nutrition.   Science 103: 326–329 (1946).
28. MELNICK, D., and OSER, B. L.   The influence of heat-processing on the functional and nutritive properties of protein.   Food Tech. III: 57–71 (1949).
29. PADER, M., MELNICK, D., and OSER, B. L.   Factors affecting the availability of lysine in heat-processed casein.   J. Biol. Chem. 172: 763–769 ('948).
30. RIESEN, W. H., CLANDININ, D. R., ELVEHJEM, C. A., and CRAVENS, W. W.   Liberation of essential amino acids from raw, properly heated, and overheated soybean oil meal.   J. Biol. Chem. 167: 143–150 (1947).
31. WESSON, L. G.   A modification of the Osborne-Mendel salt mixture containing only inorganic constituents.   Science 75: 339–340 (1932).
32. WESTFALL, R. J., BOSSHARDT, D. K., and BARNES, R. H.   Influence of crude trypsin inhibitor on utilization of hydrolyzed protein.   Proc. Soc. Exp. Biol. Med. 68: 498–500 (1948).
33. WESTFALL, R. J., and HAUGE, S. M.   The nutritive quality and the trypsin inhibitor content of soybean flour heated at various temperatures.   J. Nutrition 35: 379–389 (1948).

LABORATORY PROCEDURE FOR EVALUATING THE CURD-PRODUCING CAPACITY OF SOYA PRODUCTS [1]

Stephen J. Loska, Jr.,[2] and Daniel Melnick [3]

ABSTRACT

A procedure for the rating of soya products as sources of soy curd, employing the essential steps of Asiatic soy curd processing, involves the aqueous extraction of the soy protein and its precipitation as a curd on the addition of magnesium chloride, the curd being measured volumetrically.

Appreciably lower curd yields are obtained from material larger than that which passes through a No. 100 U. S. Standard wire sieve. Maximal solubility and precipitation of protein is obtained when the extraction is conducted at a temperature of 80°C. for a period of thirty minutes with the suspension stirred mechanically. Salts of strong acids such as calcium chloride, magnesium chloride, ferric chloride, and sodium bisulphate are effective agents for the precipitation of soy proteins. Hydrochloric acid is also an effective curding reagent. Maximal yields of soy curd using magnesium chloride as the precipitating reagent occur at pH 5.8, whereas isoelectric precipitation using hydrochloric acid is at pH 4.5. Excessive quantities of the salts used to precipitate the soy proteins have given, within the concentrations studied, smaller yields of soy curd, the decreased yields being a characteristic of the precipitating salt not necessarily correlated with the pH of the final medium.

Data are presented showing that curd volume is an accurate index to the percentage of soluble protein in the soy flours.

The use of soya in relief feeding in both Europe and the Orient created new problems in description and control of products manufactured for different purposes. In Europe soya is used as a wheat flour extender, as a meat extender, and as a major ingredient for low cost food preparations; in the Orient it is used for the manufacture of soy sauce derived from the hydrolysis of protein, for "tofu" or soy curd prepared by the precipitation of water-extractable protein, and for "miso" or soy paste.

The present specification for soya products, Joint Army-Navy Specification JAN-S-588, refers to the protein as simply $N \times 6.25$, no consideration being given to the functional or nutritional properties of the foodstuff. A product may be satisfactory for one purpose, but

fail in another. The evaluation of soya products utilized in the preparation of curd presented one of the greatest problems. This study concerns the development of a precise and rapid laboratory procedure for this purpose.

Materials and Methods

Samples of soy flour, representative of raw and heat-treated commercial products, were kindly furnished by members of the Soy Flour Association. In addition, Dr. James W. Hayward of Archer-Daniels-Midland Company, Minneapolis, Minnesota, in behalf of the Soy Flour Association, supplied data obtained in their analyses of the products according to conventional procedures.

The products consisted of (1) dehulled soy beans, (2) unheated solvent-extracted soy flour, (3) bakery type soy flour, mildly heat-processed in order to eliminate the bitter qualities of the raw bean, (4) soy flour optimally heat-processed with respect to the nutritive quality of the protein, and (5) an overheated processed flour experimentally processed for this study. These raw materials for flours were supplied in flake form. In addition, an expeller series was supplied, consisting of (6) dehulled beans which had been heat-treated, (7) expeller flour with some additional heating, (8) flour, heat-processed so the protein had optimum biological value, and (9) overheated soy flour. These samples were supplied in chip form.

Samples were found to be most easily ground with a W. J. Fitz-patrick Comminuting mill. Solvent extracted flakes were ground through a 1 mm. mesh sieve and then reground through a No. 40 wire sieve. Samples 1, 6, 7, 8, and 9, because of their appreciable fat content, were ground through a 1 mm. mesh sieve and then extracted with Skelly Solve "B" in a Soxhlet extractor for 16 hours. After thorough air-drying these were reground through a No. 40 wire sieve.

Optimum sifting time for all samples through a No. 100 U. S. Standard sieve was determined by the method of Wichser, Shellenberger, and Pence (7). All samples were sifted through a No. 100 wire sieve.

In the many soy curd methods used by the Orientals, the essential steps are (1) aqueous extraction of soluble protein, (2) separation of solution from residue, and (3) precipitation of protein by means of salts, mainly those of magnesium. The manner in which these steps are performed are many and varied but the results are comparable. When the whole bean is employed a soaking step may be utilized to remove the hulls and facilitate wet grinding. Time and temperature for extraction of the crushed or milled bean may vary widely from a few

minutes in boiling water to several hours in hot water. Separation
of solution from residue is accomplished by decantation or filtration
through a cloth. Precipitation methods employed by the Orientals
differ as to the reagent used; however, salts of calcium and magnesium,
i.e. gypsum, magnesium sulphate, calcium chloride, magnesium chlo-
ride, and mixtures derived from evaporation of sea water, are used
almost exclusively. To establish reproducible conditions for the above
steps the following basic procedure was used:

The extract was prepared by suspending 8.00 g. of soy flour in 100
ml. of distilled water, or multiple of this flour-water ratio, and stirred
with a mechanical stirring device. After extraction the suspension
was centrifuged in an International centrifuge No. 1 with trunnion
cups, radius measurement 20 cm. to the tip, 1,800 r.p.m. for ten
minutes. Twenty-five ml. of the extract liquid were pipetted into a
50 ml. long-tapered centrifuge tube graduated in fractions of ml. to
20 ml.; curding solution was added and the suspension diluted to the
50 ml. mark. Centrifuging was again accomplished at 1,800 r.p.m.
for ten minutes.

Results and Discussion

Influence of Temperature on the Extraction of the Soy Proteins.
Sample No. 3, bakery type soy flour, was selected for this study. Of
the samples submitted, it contained an intermediate quantity of
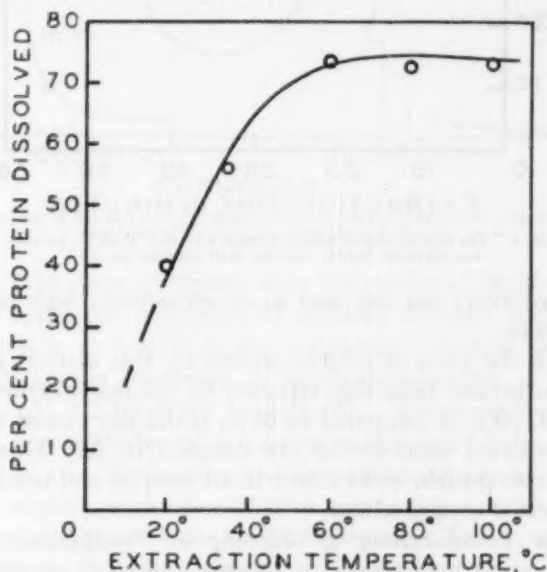
Fig. 1. The relationship of soluble protein and temperature of extraction;
time, 60 min.; test material, bakery type soy flour (Sample No. 3).

soluble protein.   The flour was extracted at several temperature levels for one hour.   Protein analyses ($N \times 6.25$) of 25 ml. aliquots of the extract, after centrifugation, indicate maximum solubility of the protein at 60°C. to 100°C. as shown in Fig. 1.   In another experiment designed to determine the effect of time, extractions were repeated at 80°C. for intervals of 10, 20, 30, 40, and 60 minutes.   Fig. 2 shows that at 20 minutes, extraction has reached a maximum with no significant change on continued heating.   Since 30 minutes and 80°C. extraction conditions are sufficient to insure maximum protein dispersion, a longer extraction period is unnecessary and undesirable because of
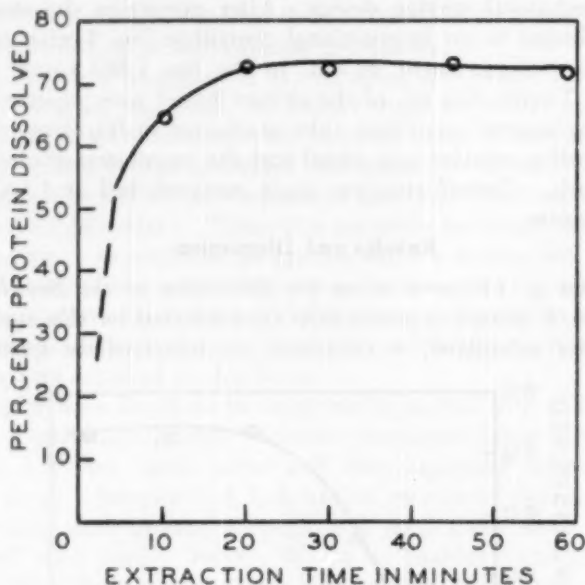
Fig. 2.   The relationship of soluble protein, extracted at 80°C., to time; test material, bakery type soy flour (Sample No. 3).

water evaporation; this can lead to an erroneously high estimate of soluble protein.

Although the yield of soluble protein by this method of hot extraction was greater than that reported by the manufacturer for this sample,[4] viz. 70% as compared to 65%, it did not exceed the manufacturer's reported value for the raw sample (No. 2).   This increased yield of soluble protein was evident in all samples and particularly in the heat treated soya products.

Optimum Concentrations of Curding or Precipitating Reagents. Several salts and hydrochloric acid were evaluated as precipitating

[4] Indirect Method; Archer-Daniels-Midland Company

agents in this study.   Sample No. 2, unheated soy flour, which exhibited greatest solubility, was used for the tests.   Protein from 25 ml. of extract was precipitated with solutions of magnesium chloride, ferric chloride, sodium bisulphate, and hydrochloric acid.   The final normality after dilution to 50 ml. was calculated.   By subtracting twice the protein content of 25 ml. of supernatant solution after precipitation and centrifugation of the curd from the protein content of the extract, the weight of protein in the curd can be determined.

Fig. 3 shows that maximal yield of precipitated protein was attained when the solutions were 0.01 N to 0.03 N with respect to the salt and
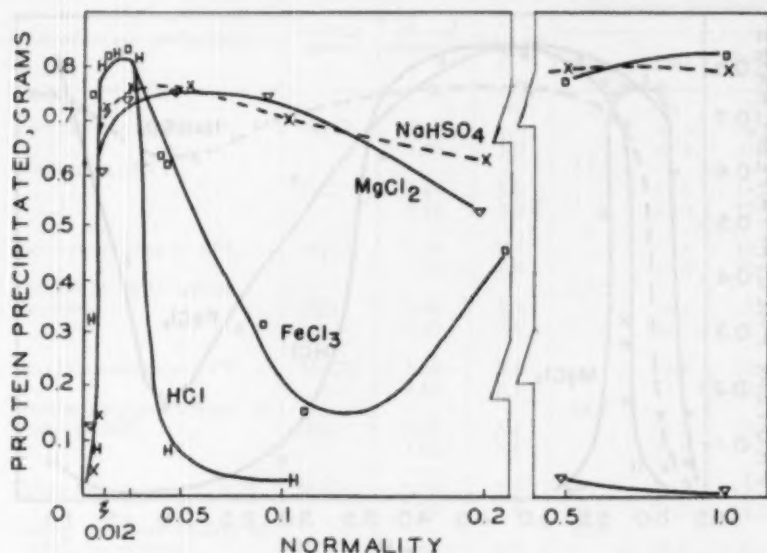
Fig. 3.   Relation of salt and acid concentration to the yield of precipitable protein of soya; test material, defatted raw soya (Sample No. 2).

acid.   This is in close agreement with the results of Smith et al. (6) who report minimum extraction of soybean nitrogen with calcium chloride solution of 0.0175 N and magnesium chloride solution of 0.025 N.   Minimum dispersion of nitrogenous matter in acid was obtained at pH 4.2 by these authors (5).   Maximum protein precipitated by hydrochloric acid was found in this study to be in the pH range of 5.0 to 4.0.   Hydrochloric acid or isoelectric precipitation was noted to be slightly more efficient than magnesium chloride, yielding approximately 4% more of the available protein.   The curves plotted in Fig. 4 indicate that the yield of curd is not necessarily correlated with the pH of the final medium.

The Effect of Heating upon the Solubility of the Protein and upon the Precipitation of the Curd. The data in Table I illustrate the effect of cold extraction and of hot extraction followed by curding from cold and hot solutions. Extraction at room temperature is not efficient under the conditions described. The curd volume was less than anticipated compared to the amount of protein in solution. The condition of the curd was fluid in nature and the residual protein after curding was large. By slightly heating the extract after the first centrifugation a more efficient precipitation was obtained, denoted by an increase in curd volume and decrease in quantity of protein in the top liquid.
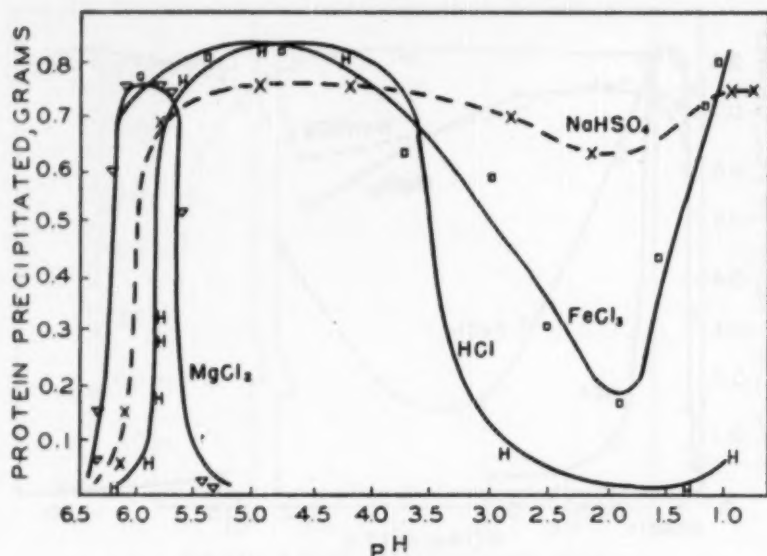
Fig. 4.   The relationship of the yield of curd to the pH of three salt solutions and hydrochloric acid; test material, defatted raw soya (Sample No. 2).

Thirty minutes extraction at 80°C. increases the yield of soluble protein (as was shown in Fig. 1), but cooling the extract at room temperature before protein precipitation gives a curd having undesirable small creamy particles which do not pack well, resulting in an apparently greater volume. Heating serves a two-fold purpose: (1) it produces the maximum yield of soluble protein in the minimum of time, and (2) causes incipient denaturation of the extractable protein, making it more readily precipitable following the addition of the curding reagent. The heat treatment yields a curd possessing good consistency and which is easily centrifuged, thereby improving the precision of the gravimetric separation.

The Effect of Granulation or Particle Size of Flour on Extraction of Protein and on Soy Curd Yield. Marked changes in curd yield by volumetric measurement were noticed with the same soy flour of different granulation. Samples of the unheated (Sample No. 2) and the optimally-heated (Sample No. 4) soy flours were used to determine the effect of granulation or particle size on the reproducibility of the

TABLE I

EFFECT OF HEATING UPON THE SOLUBILITY OF THE PROTEIN AND UPON THE PRECIPITATION OF THE CURD [1]

Extraction and curding method	Curding agent	pH curd suspension	Curd vol.	Distribution of protein [2]		
				Extract	Curd	Supernatant
			ml.	%	%	%
Two hrs. extract at 23°C.	MgCl₂	5.65	1.8		41.3	20.3
		5.65	1.9		40.1	21.6
Curding at 23°C.				61.6		
	HCl	4.80	1.9		47.6	14.0
		4.80	1.9		47.9	13.8
Two hrs. extract at 23°C.	MgCl₂	5.70	3.5		53.3	10.9
		5.70	4.0		53.3	10.9
Brought to 80°C. before curding				64.2		
	HCl	4.80	3.5		56.3	7.9
		4.80	3.5		56.1	8.1
Two hrs. extract at 23°C.	MgCl₂	5.68	5.5		53.8	10.2
		5.65	5.5		53.8	10.2
Protein solution heated 30 min. at 80°C.				64.0		
	HCl	4.60	5.5		56.7	7.3
		4.60	5.5		56.7	7.3
Thirty min. extract at 80°C.	MgCl₂	5.70	7.5		74.3	12.3
		5.70	7.5		74.3	12.3
Protein solution cooled to 23°C. for curding				86.6		
	HCl	4.60	7.0		78.2	8.4
		4.60	7.0		78.7	7.9

[1] Defatted raw soy flour (Sample No. 2) used in this study, 8 g. flour to 100 ml. water.
[2] Per cent protein calculated as per cent of total protein. Supernatant protein determined by Kjeldahl method on an aliquot of top liquid after precipitation. Protein of curd calculated by difference; extract protein minus protein of supernatant liquid after precipitation.

test. The Joint Army-Navy Specification for Soybean Products, dated May 10, 1948 specifies:

"E-7. Ninety-seven per cent of the soy flour shall pass through a U. S. Standard 100-mesh screen."

The materials through and over a 100-mesh screen of Samples No. 2 and No. 4 were tested for soluble protein and curd yield. The results in Table II, average of duplicate tests, emphasize that large differences in per cent soluble protein and in curd volume are obtainable if the

particle size of the flour is not controlled.   The per cent of protein of the initial soy flour fractions was nearly the same.   Therefore, the difference in curd yield is attributed to the greater solubility of the material passing through the 100-mesh screen, due to larger surface area and mechanical damage to cell structure.   To insure maximum solubility of protein at the time and temperature of the test all samples should be ground finer than 100-mesh.

TABLE II

EFFECT OF GRANULATION OR PARTICLE SIZE OF
FLOUR ON SOY CURD YIELD

Sample	Curd vol.	Distribution of protein[1]		
		Extract	Curd (calc.)	Supernatant
	ml.	%	%	%
Unheated soy	5.00	73.40	57.95	15.45
Sample No. 2	5.00	74.02	58.98	15.03
Over 100w	5.00	72.46	57.64	14.82
	5.00	74.22	59.19	15.03
Mean	—	73.52	58.44	15.08
Standard deviation	—	± 0.71	—	± 0.29
Unheated soy	6.25	84.90	73.08	11.82
Sample No. 2	6.25	83.85	72.02	11.82
Through 100w	6.25	85.19	73.55	11.64
	6.25	84.71	72.79	11.92
Mean	—	84.66	72.86	11.80
Standard deviation	—	± 0.55	—	± 0.12
Heated soy	1.80	27.30	17.70	9.60
Sample No. 4	1.80	27.50	18.00	9.50
Over 100w	1.80	27.30	17.70	9.60
	1.80	27.60	18.30	9.30
Mean	—	27.42	17.92	9.50
Standard deviation	—	± 0.14	—	± 0.14
Heated soy	2.30	36.24	26.95	9.29
Sample No. 4	2.30	36.06	26.77	9.29
Through 100w	2.30	35.31	26.30	9.01
	2.30	35.40	26.21	9.19
Mean	—	35.75	26.56	9.19
Standard deviation	—	± 0.47	—	± 0.13

[1] Per cent protein calculated as per cent of total protein.

Correlation of Curd Volume with Amount of Protein Precipitated. Initial determinations on the nine samples included in this study indicated a variation in soluble protein content; three samples possessed a high proportion of soluble protein while the other six had smaller amounts.   To determine if curd volume, by the proposed method of magnesium chloride precipitation, is linearly related to protein precipitated, increasing quantities of extract of Sample No. 2 were diluted to 25 ml. and subjected to the curd test.   The weight of

protein used in each case was a fraction of the amount of protein in the 25 ml. of extract.   The data plotted in Fig. 5 show that this relation is linear with a correlation coefficient of $r = + 0.996**$.

The tests were extended to 11 samples and quantities of less than 25 ml. were used whenever the smaller aliquots would yield measurable curd volumes.   For this reason aliquots less than 20 ml. were not practical in the cases of the heat-treated samples.   Fig. 6 illustrates the relationship found with 35 aliquots of 11 soy samples; a correlation coefficient of $r = + 0.984**$ and a regression equation
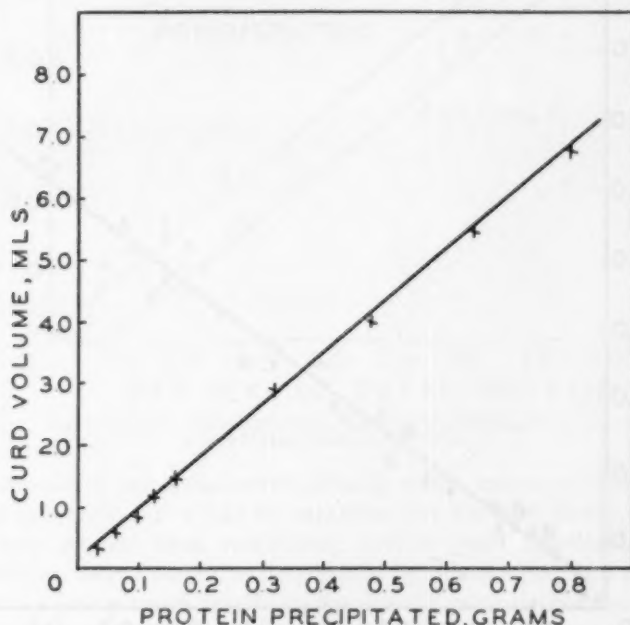
Fig. 5.   Relation of protein precipitated to curd volumes; test material, defatted raw soya (Sample No. 2).

of $Y = 7.26 X + 0.26$ were found, where Y = observed curd volume and X = grams of protein precipitated.

Included in these tests were three samples of soy meal.   These were unsolicited samples from industry without accompanying analytical data and are identified as A, B, and C.   Comparison with test samples indicates that A has been slightly heat-treated and was satisfactory for curd preparation.   Heat treatment of samples B and C materially affected the solubility of the protein so that these would have to be used in formulations where functional properties of the protein are not important.

Plotting curd volume against per cent soluble protein and per cent of protein precipitated, Fig. 7, illustrates the relationship of soluble protein to the yield of curd.   Soy flours of low protein solubility have a residual protein after precipitation of from 8 to 10%.   High protein solubility flours have a residual protein of from 10 to 14%, indicated by the dotted lines.

The data from Table II were plotted on the figure, represented by the letters Z and Y.   Letter Z represents material through a 100-mesh
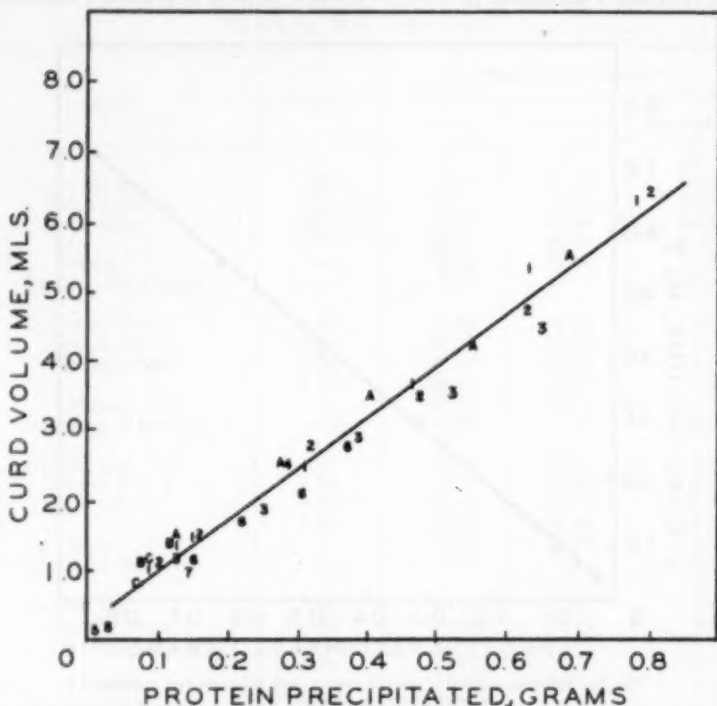
Fig. 6.   Relation of protein precipitated to curd volume with thirty-five aliquots of eleven samples of soy flour and soy meal.   The numbers refer to the soy flour test samples; the letters refer to solvent extracted soy meals.

sieve and Y the material over the wire from Samples No. 2 and No. 4. It is again evident that the yield of curd is dependent upon the amount of extractable protein.

A curd volume of not less than 4.0 ml. by this method for solvent-extracted soy flours and meals would identify such products as the type which would find acceptance with Orientals.   Alternately, heat-treated products similar to samples 4 and 5 would be useful as far as
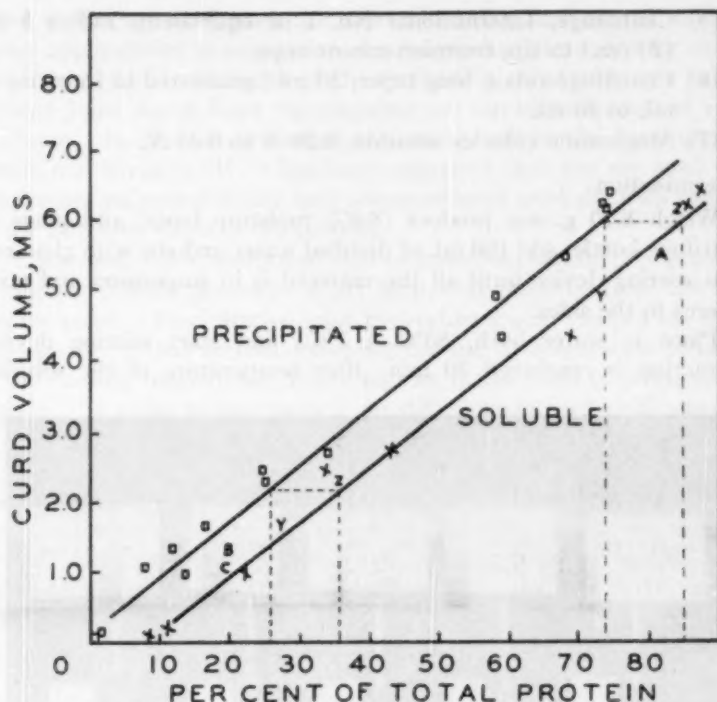
Fig. 7. Relation of per cent soluble protein in soy products and per cent of protein precipitated to curd volume.

consumption *in toto* is concerned, in soup mixes, and as protein supplements in cereals, but would be unsatisfactory for curd formation.

These studies have established that a good correlation exists between soy curd volume, as determined by this method, and quantity of protein precipitated, justifying the use of the far simpler volumetric procedure rather than the nitrogen analysis for the determination of the relative concentrations of the precipitable soy protein.

The method recommended is as follows:

Sample preparation:

Sample should be of such granulation that it passes through a No. 100-mesh U. S. Standard screen.

Apparatus:

    (1) Centrifuge bottles, Pyrex, 250 ml.

    (2) Pipettes, 25 ml.

    (3) Waterbath.

    (4) Mechanical or electrical stirring device with glass stirring rod.

(5) Centrifuge, International No. 1 or equivalent, radius 8 in. (20 cm.) to tip, trunnion mount cups.

(6) Centrifuge tubes, long taper, 50 ml., graduated in fractions of ml. to 20 ml.

(7) Magnesium chloride solution, 0.20 N to 0.25 N.

Determination:

Weigh 8.00 g. soy product (8.0% moisture basis) and place in centrifuge bottle, add 100 ml. of distilled water and stir with glass rod from stirring device until all the material is in suspension and none adheres to the sides.

Place in water bath, 80°C. ± 2°C., and start stirring device. Extraction is continued 30 min. after temperature of the solution
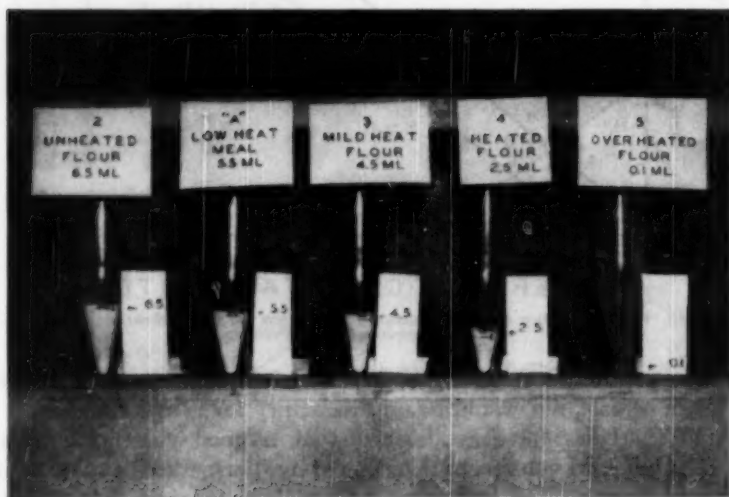
Fig. 8.   Illustration of five samples of defatted soya at the completion of the curd test.

passes 60°C. (approximately 5 min.).   Remove bottle and centrifuge at 1,800 r.p.m. for 10 min.

Pipette 25 ml. of the supernatant liquid into a long-tapered centrifuge tube, bring solution to 80°C., remove tubes and add 5 ml. of 0.2 N magnesium chloride solution while agitating the tube.   Dilute to the 50 ml. mark with water and centrifuge at 1,800 r.p.m. for 10 min. Allow centrifuge to come to rest without braking and read curd volume to the nearest fraction of a ml.   Two to three tests may be conducted on the extract for increased precision.

Fig. 8 illustrates the appearance of the centrifuge tubes at the completion of the curd test on five soybean products.   The reproduci-

bility of the soy curd test, the ease of running such assays, and its direct applicability to evaluation of soya products intended for use in the Orient, justify inclusion of the method in an amendment to the present Joint Army-Navy Specification for the identification of soya products. In current investigations conducted in the Institute by Simon and Melnick (4), it has been suggested that the soy curd test has further value in defining heat-processed soya products with respect to the nutritive value of the protein.

It was previously mentioned that magnesium chloride precipitation of the soy protein occurs at a pH somewhat removed from the isoelectric point. Precipitation with hydrochloric acid yields a soy curd comparable to that formed following the use of magnesium chloride as the curding reagent but differs slightly in the yield of precipitated protein. In order to determine if the difference in curd yield was due to some protein fraction which was precipitated by hydrochloric acid at the isoelectric point and not precipitated by magnesium chloride, electrophoretic analysis was employed. A water extract of Sample No. 2 unheated soy flour was prepared according to the test method and divided into two portions. Magnesium chloride reagent was added to one portion and hydrochloric acid to the other in concentrations which produced maximum yield of curd. Electrophoretic analyses of the protein in the two supernatant solutions, kindly conducted by Dr. A. C. Shuman, General Foods Corporation, Hoboken, New Jersey, indicated that the two solutions were identical as far as their electrophoretic mobilities were concerned and each consisted of a single component with a small degree of non-homogeneity. Thus, precipitation at the isoelectric point yields the same protein precipitate as the procedures customarily employed by the Orientals.

In this connection, it is of value to speculate on the possible fate of the antitryptic factor in the raw or mildly-heated soya products which are of the type most favorable for soy curd formation. Such mildly-heated products contain the antitryptic factor, which has been held to be responsible for poor protein-utilization *in vivo* (2, 3). Precipitation with hydrochloric acid at the isoelectric point has been employed (1, 3) to remove irrelevant protein leaving in solution the antitryptic complex. It seems quite likely from the electrophoretic studies that the magnesium chloride reagent would also be effective for this purpose. However, the efficiency with which the antitryptic complex can be isolated by these procedures has not been adequately studied. The possibility of co-precipitation of the antitryptic complex with the soy curd does exist. This point of importance in predicting the value of the soy curd as a source of protein of high biological value is now under investigation in our laboratories.

Literature Cited

1. BORCHERS, R., ACKERSON, C. W., MUSSEHL, F. E., and MOEHL, A.   Trypsin inhibitor, VIII.   Growth inhibiting properties of a soybean trypsin inhibitor. Arch. Bioch. **19**: 317–322 (1948).
2. HAM, W. E., SANDSTEDT, R. M., and MUSSEHL, F. E.   The proteolytic inhibiting substance in the extract from unheated soybean meal and its effect upon growth in chicks.   J. Biol. Chem. **161**: 635–642 (1945).
3. KLOSE, A. A., HILL, B., and FEVOLD, H. L.   Presence of a growth inhibiting substance in raw soybeans.   Proc. Soc. Exp. Biol. Med. **62**: 10–12 (1946).
4. SIMON, M., and MELNICK, D.   The *in vitro* digestibility of raw and heat-processed soy products varying in the nutritive value of the protein.   Cereal Chem. **27**: 114–126 (1950).
5. SMITH, ALLEN K., CIRCLE, S. J., and BROTHER, G. H.   Peptization of soybean proteins.   The effect of neutral salts on the quantity of nitrogenous constituents extracted from oil-free meal.   J. Am. Chem. Soc. **60**: 1316–1320 (1938).
6. SMITH, ALLEN K., and CIRCLE, S. J.   Peptization of soybean proteins.   Extraction of nitrogenous constituents from oil-free meal by acids and bases with and without added salts.   Ind. Eng. Chem. **30**: 1414–1418 (1938).
7. WICHSER, F. W., SHELLENBERGER, J. A., and PENCE, R. O.   Relationship of physical properties of wheat flour to granulation.   Cereal Chem. **24**: 381–393 (1947).

DETERMINATION OF IRON IN CEREALS, FLOUR, AND BREAD [1]

CHESTER R. SZALKOWSKI with H. A. FREDIANI [2]

ABSTRACT

To determine iron in flour and bread, muffle ignition of a sample moistened with magnesium nitrate was found to be preferable to other ashing procedures because it prevents the loss of iron and offers simplicity and ease of manipulation.   Results are reproducible to within 0.5%.

Possible interference by pyrophosphate is overcome by the presence of considerable quantities of magnesium, and by acid hydrolysis.   Intensity and stability of the thiocyanate color complex with iron is increased by extraction with normal butyl alcohol.

Analyses were carried out on flour, bread, corn meal, macaroni, corn grits, and other cereal products.   The initial iron content of the sample was ascertained and in each case additional iron was added and the recovery measured.   In all instances this was found to be within 2.5% of the theoretical recovery.   In some instances, addition of phosphate was also carried out, and in no event did this additional phosphate interfere with complete iron recovery.

Since iron is an important constituent of foodstuffs and is included in some form in the enrichment of cereals, flour, and bread, an analytical method for its determination in relatively small quantities is essential.   Both the Association of Official Agricultural Chemists and the American Association of Cereal Chemists have taken cognizance

of the problem involved by the publication of thoroughly tested methods for this analysis (2, 12).

In the official A.A.C.C. method specific for iron in flour, semolina and related products, 20 g. of unenriched flour or 5 g. of enriched flour, whole wheat or bread (2) are ashed overnight in a platinum dish at 550–600°C., the ash is dissolved in HCl, the iron reduced by hydroquinone and the ferrous iron then determined photocolorimetrically by α, α′-dipyridyl.

In the A.O.A.C. method for iron in cereal foods (12) a 10 g. sample is ashed in a platinum, silica, or porcelain dish at about 550°C., the ash is dissolved in HCl, reduced with hydroxylamine and the iron measured photometrically with either orthophenanthroline or α, α′-dipyridyl. In this method, except for self-rising flour or bread, an ashing aid such as magnesium nitrate solution or nitric acid is recommended to be added to the initial ash if it is not obtained free of carbon. A recent report by Munsey (13) has confirmed the applicability of the A.O.A.C. method to the determination of iron in enriched spaghetti, enriched degerminated corn meal, and enriched corn meal.

During the past five years or more a generally applicable method based on the formation of ferric thiocyanate has been under investigation in our laboratories and has been found to yield results strictly in accordance with the two official methods outlined above. This method embodies certain modifications which are primarily time saving in nature. It has been investigated on commercially available samples of unenriched and enriched flour, self-rising flour, phosphated flour, whole wheat and rye flours, white, rye and whole wheat breads, enriched hominy grits, flaked grits, corn meal, a malted wheat cereal, doughnuts, doughnut mix, and macaroni. Interference of both calcium and phosphorus are circumvented.

A review of the literature reveals that the thiocyanate reagent is very widely used in the determination of small amounts of iron. The vast information on its use is in some cases contradictory. This reagent is particularly well suited for use on samples prepared with a high acid concentration, and was chosen for this reason. According to Woods and Mellon (21) the following variables must be kept reasonably constant: (a) amount and kind of acid, (b) excess quantity of oxidizing agent, (c) time of standing, (d) presence of interfering ions, and (e) dielectric constant of solvent.

Hallinan (7) has shown that a high concentration of hydrochloric acid is exceptionally well suited for the development of the ferric thiocyanate complex. Extraction by an immiscible solvent is of importance when small quantities of iron are to be determined. Isoamyl (15), isobutyl (17), and normal butyl alcohols appear to be ideal

solvents for concentrating, intensifying and stabilizing the color. The ferric thiocyanate complex extracted with isobutyl alcohol has a maximum absorption at 485 mμ; with normal butyl alcohol at 490 mμ and the latter extraction is free from disturbances which occur when isoamyl alcohol is used (3). Winsor (19) has shown that the dielectric constant is satisfactory for the dissociation of the solute.

Materials and Methods

Apparatus included: (1) A Coleman Double Monochromator and a Coleman Universal Spectrophotometer. (2) Pyrex Glassware—All glassware was cleaned with nitric acid and thoroughly rinsed with distilled water. (3) Silica Dishes—100 ml. silica dishes were cleaned with nitric acid and rinsed with distilled water.

Reagents consisted of: (1) A.C.S. reagent grade nitric and hydro-chloric acids, potassium thiocyanate, and magnesium nitrate. Normal butyl alcohol, reagent grade, b.p. 116–118° was used without further treatment. (2) Butanolic potassium thiocyanate solution—100 g. of reagent grade potassium thiocyanate were dissolved in 100 ml. of water, heated to 25–30°C., diluted to 1000 ml. with n-butyl alcohol, shaken vigorously for at least 15 minutes and allowed to clear. (3) Magnesium nitrate—55 g. reagent grade magnesium nitrate, $Mg(NO_3)_2 \cdot 6H_2O$, were dissolved in sufficient distilled water to make 100 ml. of solution. (4) A stock standard iron solution was made by weighing 1.000 g. of reagent iron wire into a clean, iron-free beaker, dissolved in 20% hydrochloric acid to which 2 ml. of concentrated nitric acid were added. The solution was then carefully evaporated to dryness (on steam bath) and dissolved in 20 ml. of 10% hydro-chloric acid, quantitatively transferred to a 1000 ml. volumetric flask and diluted to volume. This stock solution contained 1 mg. iron per ml. and was stable for relatively long periods of time when kept in a pyrex bottle. (5) The working standard was prepared by diluting 10 ml. of the stock solution to exactly 100 ml. with distilled water. One ml. contained 0.1 mg. iron (Fe).

A sufficient amount of finely powdered sample to contain about 0.1 mg. of iron was weighed into a 100 ml. silica dish. Exactly 10 ml. of magnesium nitrate solution were added and the mixture stirred with a glass rod until a uniform paste was obtained. The paste was spread in the dish and the rod wiped with a small piece of ashless filter paper, the latter being added to the sample. The paste was covered with about 5 ml. of alcohol and the dish transferred to a muffle furnace set at 600–650°C. The door of the furnace was left slightly open. When the ash turned entirely white or grayish-white (45 to 60 minutes), the dish was removed from the furnace and cooled. The ash was

carefully moistened with a fine stream of water and 3 ml. of nitric acid added and then evaporated to dryness on the steam bath. The ash was redissolved in a few ml. of water, 10 ml. of hydrochloric acid added and again evaporated on the steam bath almost to dryness. The residue was dissolved in 15 ml. 1 N hydrochloric acid and heated on the steam bath for about 15 minutes then cooled, transferred to a 50 ml. volumetric flask and diluted to volume with 1 N hydro-
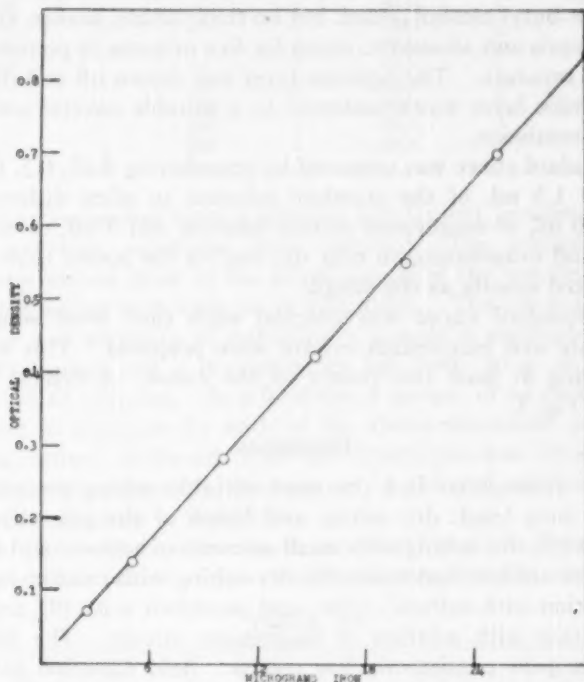
FIG. 1

chloric acid. If the resultant solution was not clear, it was filtered through a small filter, the first 15 ml. of filtrate being rejected.

A 10 ml. aliquot was transferred to a 60 ml. glass stoppered separatory funnel and exactly 15 ml. of butanolic potassium thiocyanate solution added, then shaken vigorously for 30 seconds and allowed to stand for five minutes to permit the two phases to separate. The aqueous layer was drawn off and discarded. The funnel was inverted and slowly revolved so as to dislodge any water particles that clung to the walls of the funnel and permitted to stand for five minutes. The small amount of water which separated from the alcohol was drawn

off and the alcohol layer transferred to a suitable cuvette. Readings were made at 490 mμ, with the blank set at 100% transmission. The separation of the two phases was carefully controlled. The ratio of alcohol to water after the first separation became very great, and at this point a more than gentle revolving of the funnel produced an emulsion. This resulted in a slow separation of water and caused erratic results. Centrifuging corrected this difficulty.

A 10 ml. aliquot was transferred to a 60 ml. glass-stoppered funnel, 15 ml. of n-butyl alcohol added, but no thiocyanate, shaken vigorously for 30 seconds and allowed to stand for five minutes to permit the two phases to separate. The aqueous layer was drawn off and discarded. The alcoholic layer was transferred to a suitable cuvette and set for 100% transmission.

A standard curve was prepared by transferring 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 ml. of the standard solution to silica dishes, adding exactly 10 ml. of magnesium nitrate solution and 3 ml. of nitric acid to each and evaporating to near dryness on the steam bath. These were treated exactly as the sample.

The standard curve was checked each time fresh solutions of thiocyanate and magnesium nitrate were prepared. This was done by verifying at least two points on the curve. A typical curve is shown in Fig. 1.

Discussion

In an attempt to find the most suitable ashing procedure, the following were tried: dry ashing and fusion of the ash with sodium carbonate (1), dry ashing with small amounts of sulfuric acid (10), dry ashing with sodium hydroxide (8), dry ashing with calcium carbonate (6), digestion with sulfuric, nitric, and perchloric acids (9), and ashing after wetting with solution of magnesium nitrate. The first three procedures gave consistently low results. Acid digestion gave satisfactory results after a long and tedious operation. It was found that if the sample of flour, bread, or cereal was thoroughly mixed to form a

TABLE 1

Iron Recovered from Enriched * Patent Flour
(Mg. per pound)

Dry ashing and Na₂CO₃ fusion	CaCO₃ ashing	H₂SO₄ ashing	NaOH ashing	Wet ashing	Mg(NO₂)₂ ashing
9.27	13.05	11.40	12.60	13.60	13.96
11.44	12.85	13.00	12.05	13.75	13.73
10.48	13.28	10.80	13.02	13.85	13.88

* Enriched to contain 13 mg. iron per pound.

TABLE II

IRON RECOVERY IN PRESENCE OF PYROPHOSPHATE
20 μg. Fe and 50 mg. P_2O_5 (added as $Na_4P_2O_7$)
(Solutions made up in $N/1$ HCl)

Magnesium present	Aluminum present	Fe recovered before hydrolysis	Fe recovered after hydrolysis
mg.	mg.	%	%
0	0	12.5	94.5
50	—	50.5	98.0
100	—	70.0	98.6
250	—	93.0	98.6
350	—	93.5	98.5
	10	86.5	97.0
	50	97.0	98.6
	100	98.5	98.6
	200	98.5	98.5

paste with magnesium nitrate solution and ignited, consistently good checks and iron recoveries were obtained. The addition of the magnesium nitrate prior to the initial ashing of the sample permits a more rapid ashing with little or no difficulties. The ash is always free of carbon and necessity of reigniting a sample is eliminated. Silica dishes were used and a grayish-white ash from 10 g. of flour was obtained in 45 minutes. As a final test a sample of an enriched flour was ashed in triplicate by each of the above-mentioned procedures. The iron content of the ash after acid hydrolysis was determined by extraction with potassium thiocyanate in n-butyl alcohol solution. The results are given in Table I.

After numerous experiments, it was found that the formation and extraction of the color complex could be accomplished in one operation

TABLE III

Sample	A.O.A.C. mg. Fe/lb. found	KCNS mg. Fe/lb.	Proposed ignition and α, α'-dipyridyl
Enriched flour	14.0	14.0	14.3
Enriched self-rising flour (A)	19.3 19.8	19.7 19.4	19.5
Enriched self-rising flour (B)	13.0 12.6	12.7 12.8	13.2
Enriched corn meal	20.6 20.0	20.3 20.0	20.6
Macaroni	19.0 18.0	18.7 18.9	18.4
Corn grits	21.2 21.8	21.1 21.4	21.2

by shaking the acidified solution of iron with a solution of potassium thiocyanate in normal butyl alcohol. Normal butyl alcohol was selected over isobutyl and isoamyl alcohols because of its ability to dissolve greater quantities of the thiocyanate. This solvent has a relatively low dielectric constant and the resulting solution of the color complex is stable for several hours.

Flour and bread ash contain small amounts of pyrophosphates, and in the manufacture of enriched and self-rising flours, phosphates, and/or pyrophosphates are often added. On ignition, the phosphates are converted to pyrophosphates which have been shown to interfere seriously with the colorimetric determination of iron, owing to the formation of stable complexes (5, 11, 14, 21). The inhibitory effect of pyrophosphate can be eliminated by employing so-called "wet

TABLE IV

IRON RECOVERED FROM FLOUR, BREAD, ETC.

	mg. P_2O_5 per g. added as Na_2HPO_4	mg. P_2O_5 per g. added as $Na_4P_2O_7$	mg. P_2O_5 per g. added as $CaHPO_4$	Iron present* μg./g.	Iron added μg./g.	Total iron present μg./g.	Total iron recovered μg./g.	% Recovery
Unenriched flour	—	—	—	7.9	10.0	17.9	18.1	101.0
	—	—	—	7.9	10.0	17.9	17.8	99.4
	—	—	—	7.9	15.0	22.9	23.2	101.3
	—	—	—	7.9	20.0	27.9	27.4	98.2
	50	—	—	7.9	15.0	22.9	22.9	100.0
	100	—	—	7.9	15.0	22.9	22.3	97.4
	25	—	—	7.9	—	7.9	7.8	98.7
	—	25	—	7.9	—	7.9	7.8	98.7
	—	25	—	7.9	15.0	22.9	22.5	98.3
	—	—	25	7.9	—	7.9	7.7	97.5
	—	—	25	7.9	15.0	22.9	23.2	101.3
Unenriched self-rising flour	—	—	—	9.8	20.0	29.8	29.4	98.7
	—	—	—	9.8	20.0	29.8	29.1	97.7
	—	—	—	9.8	50.0	59.8	58.4	97.7
Enriched flour	75	—	—	30.8	—	30.8	29.4	95.5
	25	—	—	30.8	—	30.8	29.8	96.8
	25	—	—	30.8	—	30.8	30.0	97.4
	—	—	30	30.8	—	30.8	30.0	97.4
	—	25	—	30.8	—	30.8	30.1	97.7
	—	50	—	30.8	—	30.8	30.1	97.7
Enriched 100% self-rising flour	—	25	—	30.7	—	30.7	30.2	98.4
Enriched 85% extraction self-rising flour	—	—	—	30.0	10.0	40.0	39.9	99.8
	—	25	—	30.0	—	30.0	29.4	98.0
	—	25	—	30.0	10.0	40.0	39.3	98.3
Enriched 60% extraction self-rising flour	—	—	—	28.9	10.0	38.9	39.0	100.3

* Determined by this method on materials used prior to addition of Na_2HPO_4 and Fe.

TABLE IV (Continued)

mg. P₂O₅ per g. added as NaH₂PO₄	mg. P₂O₅ per g. added as Na₄P₂O₇	mg. P₂O₅ per g. added as CaHPO₄	Iron present* μg./g.	Iron added μg./g.	Total iron present μg./g.	Total iron recovered μg./g.	% Recovery
—	25	—	28.9	—	28.9	38.3	97.9
—	25	—	28.9	10.0	38.9	38.4	98.7
Enriched 40% extraction flour, self-rising							
—	—	—	29.8	10.0	39.8	39.7	99.7
—	25	—	29.8	—	29.8	29.5	98.3
—	25	—	29.8	10.0	39.8	39.3	98.7
75% patent enriched phosphate flour							
—	—	—	31.6	10.0	41.6	41.3	99.3
—	—	—	31.6	20.0	51.6	51.1	99.0
Whole wheat flour							
—	25	—	42.2	—	42.2	41.7	98.8
—	25	—	42.2	10.0	53.2	52.8	99.2
Rye flour (whole)							
—	25	—	11.9	—	11.9	11.6	97.5
—	25	—	11.9	10.0	21.9	21.7	99.1
Enriched white bread							
—	25	—	40.8	—	40.8	40.2	97.6
—	25	—	40.8	10.0	50.8	50.2	98.8
Whole wheat bread							
—	25	—	51.0	—	51.0	50.5	99.0
—	25	—	51.0	10.0	61.0	50.5	99.2
Bread							
—	25	—	50.8	—	50.8	50.4	99.2
—	25	—	50.8	10.0	60.8	60.5	99.5
—	25	—	50.8	—	50.8	48.9	96.2
Enriched hominy grits							
—	25	—	30.6	—	30.6	30.0	98.0
—	25	—	30.6	10.0	40.6	40.1	98.8
Flaked grits							
—	—	—	20.5	15.0	35.5	34.7	97.7
—	25	—	20.5	15.0	35.5	34.7	97.7
Corn meal							
—	—	—	38.4	15.0	53.4	52.8	98.8
—	25	—	38.4	15.0	53.4	52.3	98.0
Malted wheat cereal							
—	—	—	37.7	15.0	52.7	51.8	98.3
—	25	—	37.7	15.0	52.7	51.3	97.3
Doughnuts							
—	—	—	15.2	10.0	25.2	25.0	99.3
—	25	—	15.2	10.0	25.2	24.0	95.2
Doughnut mix							
—	—	—	16.3	10.0	26.3	25.9	98.5
—	25	—	16.3	10.0	26.3	25.6	97.5

ashing" or acid digestion procedures (17), which prevent the formation of pyrophosphate or by hydrolysis of the ash, prior to color development. Hydrolysis can be effected by heating either with acids or alkali (4, 16).

It is to be expected that the interference with the thiocyanate color complex formation, by pyrophosphate, would be decreased by the presence of considerable quantities of any other ion which forms a complex with the pyrophosphate. Monovalent ions have an effect on the inhibiting action of pyrophosphate, divalent ions have an appreciable effect and trivalent positive ions have a very pronounced effect. Aluminum and zirconium have been suggested to liberate iron from its complexes with phosphates (18, 20). Experiments showed

that only 12% of the iron is recoverable directly from 10 ml. of a 1 N hydrochloric acid solution, containing 20 μg. of iron and 50 mg. of P_2O_6 (added as $Na_4P_2O_7$). Hydrolysis of this solution increased the recovery to 94.5%. The presence of 250 mg. magnesium and hydrolysis increased the experimental recovery to 98.5%. In the presence of 10 mg. of aluminum the experimental recovery was 86.5% and in presence of 100 mg. of aluminum was found to be 98.5%. Table II shows the percentage of iron recovered from solution containing magnesium and aluminum before and after hydrolysis.

It is apparent that smaller amounts of aluminum are as effective as larger amounts of magnesium in overcoming interference of pyrophosphate. Since, in conjunction with hydrolysis, magnesium is just as effective as aluminum, the former was selected for the ashing procedure, because the ash obtained is readily soluble in acid while that of aluminum is practically insoluble.

In Table III are compared a series of samples that have been analyzed by the official A.O.A.C. method and the method herein described. The values obtained by both methods are well within the limits of experimental error.

Analyses carried out on many cereal products are listed in Table IV. In these analyses typical commercial samples were investigated. The initial iron content of the sample was ascertained and in each case additional iron added and the recovery measured. In all instances this was found to be within 2.5% of the theoretical recovery. In some instances, addition of phosphate was also carried out, and in no event did this additional phosphate interfere with complete iron recovery.

Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods. Am. Assoc. Cereal Chemists. St. Paul, Minn. (1941).
2. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (5th Edition). Am. Assoc. Cereal Chemists. St. Paul, Minn. (1947).
3. DANIEL, H. A., and HARPER, H. J. A modified sulfocyanate procedure for the determination of small quantities of iron. J. Assoc. Official Agr. Chem. 17: 286–290 (1934).
4. ELVEHJEM, C. A. A note on the determination of iron in milk and other biological materials. J. Biol. Chem. 86: 463–467 (1930).
5. ELVEHJEM, C. A., and HART, E. B. Iron in nutrition. Quantitative methods for the determination of iron in biological materials. J. Biol. Chem. 67: 43–51 (1926).
6. FARRAR, G. E. The determination of iron in biological materials. J. Biol. Chem. 110: 685–694 (1935).
7. HALLINAN, F. J. Determination of iron in water. Ind. Eng. Chem., Anal. Ed. 15: 510–512 (1943).

8. Hoffman, C., Schweitzer, T. R., and Dalby, G.   Iron content of bread and bread ingredients.   Ind. Eng. Chem., Anal. Ed. 12: 454–456 (1940).
9. Jackson, S. H.   Determination of iron in biological material.   Ind. Eng. Chem., Anal. Ed. 10: 302–304 (1938).
10. Klumpp, T. G.   The determination of iron in biological materials.   J. Biol. Chem. 107: 213–223 (1934).
11. Leeper, G. N.   Notes on the thiocyanate method of estimating iron.   Influence of different classes of phosphates.   Analyst 55: 370–371 (1930).
12. Association of Official Agricultural Chemists.   Official and Tentative Methods of Analysis (6th Edition).   Assoc. Official Agr. Chemists.   Washington, D. C. (1946).
13. Munsey, V. E.   Report on calcium in enriched bread.   J. Assoc. Official Agr. Chem. 29: 276–278 (1946).
14. Peters, C. A., and French, C. L.   A study of the ferric thiocyanate reaction.   Ind. Eng. Chem., Anal. Ed. 13: 604–607 (1941).
15. Stokes, H. N., and Cain, J. R.   On the colorimetric determination of iron. With special reference to chemical reagents.   J. Am. Chem. Soc. 27: 409–443 (1907).
16. Stugart, R.   Determination of iron in milk and other biological material.   Ind. Eng. Chem., Anal. Ed. 3: 390–393 (1931).
17. Thompson, J. B.   Determination of iron in food products.   Ind. Eng. Chem., Anal. Ed. 16: 646–648 (1944).
18. Vanossi, R.   Determination of ferric iron by thiocyanate.   Anales asoc. quim. argentina 29: 48–74 (1941).
19. Winsor, H. W.   Intensity and stability of ferric thiocyanate color.   Ind. Eng. Chem., Anal. Ed. 9: 453–455 (1937).
20. Wirth, H. E.   Colorimetric determination of small amounts of metaphosphate and pyrophosphate.   Ind. Eng. Chem., Anal. Ed. 14: 722–725 (1942).
21. Woods, J. T., and Mellon, M. G.   Thiocyanate method for iron.   Ind. Eng. Chem., Anal. Ed. 13: 551–554 (1941).

THE NIACIN AND PANTOTHENIC ACID CONTENT OF CERTAIN OHIO CORN HYBRIDS [1]

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ABSTRACT

The results of two years' study with eight corn hybrids grown each year in seven different locations in the state of Ohio show that Ohio W36 was significantly higher in niacin than any other corn hybrid studied, while Ohio C38 and Ohio M34 were significantly higher in niacin than Ohio K24, U.S. 13, U.S. 379, and Ohio M20; and Ohio C12 was significantly higher in niacin than U.S. 13, U.S. 379, and Ohio M20; while Ohio K24 was higher in niacin than U.S. 379 and Ohio M20.

The data show that Mahoning County grew corn of the highest niacin content.   Paulding, Belmont, and Madison Counties are locations where high niacin corn was produced, but not equal to that of Mahoning, while Hamilton County produced corn of the lowest niacin content.

The results also show that Ohio W36 and Ohio M34 have the highest pantothenic acid content of the eight corn hybrids studied, and U.S. 379 and U.S. 13 contained the lowest amount.   The other four corn hybrids are intermediate in pantothenic acid content.

Pantothenic acid content also appears to be highest in corn hybrids grown in Mahoning County.   Belmont County, however, produced corn of a similar (high) pantothenic acid content as that of Mahoning County, while Miami and Madison Counties produced corn of the lowest pantothenic acid content.   Hamilton County was also a producer of corn of low pantothenic acid content.   The other counties are intermediate between the high and low area producers.

The data show that heredity plays an important role in the niacin and pantothenic acid content of corn and that both vitamins are affected by environmental factors, such as soil and weather, which confirms our previous (Hunt *et al.*, 1947, and Ditzler *et al.*, 1948) observations.

Previous work (Hunt *et al.*, 1947) had shown that the niacin and pantothenic acid content of nine different corn hybrids grown in five corn belt states in three different years varied significantly.   In a later study (Ditzler *et al.*, 1948) it was shown that the niacin and pantothenic acid contents were due to the genetic makeup of the hybrids and to the environment (location and season).

The present study was undertaken to obtain further information on niacin and pantothenic acid content of some of the principal double-cross corn hybrids grown in various parts of the state over a period of three years.   The 1947 samples were immature, due to late planting and early frost, and therefore the data are not included in this report.

Materials and Methods

Eight double-cross hybrids grown at seven locations in Ohio during two crop years were included in this study.   The corn hybrids studied were Ohio W36, Ohio C38, Ohio M34, Ohio M20, Ohio C12, Ohio K24, U.S. 13, and U.S. 379, and the locations were Mahoning, Madison, Miami, Belmont, Paulding, Wayne, and Hamilton Counties.

The representative samples were obtained by taking the grain from two kernel rows from twelve ears and replicating five times.   The final sample was a composite of the above material.   The samples were ground to a fine powder and stored in air-tight containers in a dark room.   Niacin and pantothenic acid were assayed microbiologically, using methods described in a previous study (Hunt *et al.*, 1947).   The niacin and pantothenic acid contents, expressed in μg. per g., were calculated to a moisture-free basis.   The data for the eight hybrids were subjected to the analysis of variance test to determine the significance of variation due to the genetic makeup of the hybrids, location where grown and the year during which the crop was grown, as well as interactions of these three primary sources of variation.

Results and Discussion

The niacin and pantothenic acid contents of the corn hybrids are shown in Tables I and II.   It is observed that there is little difference

TABLE I

NIACIN CONTENT OF OHIO CORN HYBRIDS

(µg. per g.—Moisture-free basis)

Variety of hybrid	1945 Location (county)								1946 Location (county)								Two-year average and standard error
	Wayne	Mahoning	Belmont	Paulding	Miami	Madison	Hamilton	Average	Wayne	Mahoning	Belmont	Paulding	Miami	Madison	Hamilton	Average	
Ohio C12	23.1	23.1	23.1	23.3	22.4	22.0	22.4	22.8	24.4	22.2	22.4	25.3	24.1	23.0	21.0	23.2	23.0±.3
Ohio C38	21.3	26.0	26.9	24.4	20.9	24.9	20.9	23.7	22.4	21.8	25.1	27.7	23.3	24.9	20.5	23.7	23.7±.6
Ohio K24	22.0	20.2	21.6	24.0	20.1	25.5	21.3	22.1	21.1	24.2	22.2	23.2	22.5	24.7	21.4	22.8	22.4±.4
Ohio M20	21.9	23.0	24.2	21.9	20.2	22.8	19.8	22.0	20.1	25.7	21.3	19.8	20.7	19.7	18.1	21.0	21.5±.5
Ohio M34	24.1	21.7	24.1	26.7	24.1	26.0	23.3	24.3	20.9	28.3	22.1	23.3	22.6	22.2	21.3	23.0	23.6±.5
U. S. 13	19.1	23.0	21.9	21.8	21.3	21.8	20.1	21.3	20.6	27.2	20.5	22.1	22.6	22.6	20.2	22.2	21.7±.5
U. S. 379	17.0	23.9	22.1	24.5	22.1	23.1	20.4	21.9	20.8	25.4	20.4	21.2	21.1	19.8	17.7	20.9	21.4±.6
Ohio W36	24.9	27.2	30.1	29.5	24.3	25.9	24.9	26.6	24.7	24.0	22.7	25.9	24.5	24.2	23.0	24.1	25.3±.5
Average	21.7	23.7	24.7	24.9	22.1	24.4	21.7	23.3	21.9	25.0	22.2	23.7	22.5	21.9	20.3	22.5	22.5±.2
Two-year average	21.8	24.3	23.5	24.3	22.3	23.1	21.0										

Minimum significant difference (amount by which a pair of two-year averages must differ to be significant from each other):

Location, 0.8, significant; 1.0, highly significant.

Hybrid,   0.8, significant; 1.1, highly significant.

TABLE II

PANTOTHENIC ACID CONTENT OF OHIO CORN HYBRIDS

(μg. per g.—Moisture-free basis)

Variety of hybrid	1945								1946								Two-year average and standard error
	Location (county)								Location (county)								
	Wayne	Maho-ning	Bel-mont	Paul-ding	Miami	Madi-son	Hamil-ton	Aver-age	Wayne	Maho-ning	Bel-mont	Paul-ding	Miami	Madi-son	Hamil-ton	Aver-age	
Ohio C12	4.8	4.2	6.1	3.7	3.3	3.2	4.2	4.2	3.7	6.2	3.9	6.2	3.0	2.8	3.0	4.1	4.2±.3
Ohio C38	5.2	3.7	6.7	4.1	3.4	3.6	4.3	4.4	3.6	4.7	2.7	5.6	3.8	4.7	3.9	4.2	4.3±.3
Ohio K24	4.2	3.2	6.8	3.9	4.9	3.3	4.9	4.4	4.0	5.2	2.8	4.8	4.2	5.3	4.9	4.4	4.4±.3
Ohio M20	3.8	3.9	5.9	3.6	3.2	3.4	3.7	3.9	3.8	6.1	3.4	4.7	5.6	4.6	4.1	4.6	4.3±.2
Ohio M34	4.3	4.0	6.3	4.9	3.7	4.1	5.1	4.7	4.6	7.2	3.9	5.6	4.2	4.9	4.9	5.0	4.9±.3
U.S. 13	4.6	4.1	4.1	2.3	2.7	2.1	2.6	3.2	4.0	6.1	2.9	4.6	3.8	4.2	4.2	4.2	3.7±.3
U.S. 379	4.0	4.1	8.6	3.2	2.4	1.9	3.0	3.9	3.1	5.4	2.9	4.9	3.4	4.2	2.6	3.8	3.9±.4
Ohio W36	5.1	2.8	7.2	3.3	3.0	4.7	4.6	4.3	5.6	8.6	3.7	6.3	4.4	5.9	3.7	5.4	4.9±.4
Average	4.6	3.7	6.4	3.8	3.2	3.2	4.0	4.1	4.0	6.1	3.2	5.1	4.0	4.4	3.8	4.4	4.3±.1
Two-year average	4.3	4.9	4.8	4.4	3.6	3.8	3.9	4.3									

Minimum significant difference (amount by which a pair of two-year averages must differ to be significant from each other):

     Location, 0.5, significant; 0.7, highly significant.

     Hybrid, 0.5, significant; 0.6, highly significant.

TABLE III.

The Niacin and Pantothenic Acid Content of Corn Inbreds (Parents of Corn Hybrids)
(µg. per g.—Moisture-free basis)

Hybrid	Genetic makeup (inbred lines)	Niacin	Av.	Pantothenic acid	Av.
Ohio C12	(Ind.WF9×Oh.07) (Ill.Hy×Ia.L317)	25.8×27.0 15.8×12.4	20.2	8.2×5.8 3.3×4.1	5.3
Ohio C38	(Ind.WF9×Hy) (Oh.40B×Oh.02)	25.8×15.8 13.6×32.0	21.8	8.2×3.3 7.3×6.9	6.4
Ohio K24	(Oh.51A×WF9) (Oh.33×Oh.40B)	23.0×25.8 13.3×13.6	18.9	5.5×8.2 4.6×7.3	6.4
Ohio M20	(Oh.26×Oh.51) (Oh.33×Oh.40B)	15.8×16.8 13.3×13.6	14.9	6.6×6.2 4.6×7.3	6.2
Ohio M34	(Oh.26×Oh.51) (Oh.40B×Oh.02)	15.8×16.8 13.6×32.0	19.5	6.6×6.2 7.3×6.9	6.7
U.S. 13	(Ill.Hy×Ia.L317) (Ind.WF9×Ind.38-11)	15.8×12.4 15.8×20.6	18.6	3.3×4.1 8.2×6.2	5.4
U.S. 379	(Ill.Hy×C17) (Ia.P8×T8)	15.8×24.7 17.8×19.1	19.8	3.3×8.1 5.8×6.1	5.8
Ohio W36	(Oh.51A×Ind.WF9) (Oh.40B×Oh.02)	23.0×25.8 13.6×32.0	23.6	5.5×8.2 7.3×6.9	7.0

in the average niacin and pantothenic acid content for each corn hybrid from all locations due to year, but there is considerable difference in the vitamin content of each hybrid from one location to another.   This shows that these two vitamins can be influenced by soil and other environmental factors.   These differences are greater, on a percentage basis, in the case of pantothenic acid than in the case of niacin.

For comparative purposes, the genetic makeup of the corn hybrids with niacin and pantothenic acid content of each, and the inbreds [3] of which each hybrid is compared, are given in Table III.

The results of statistical analysis show that Ohio W36 is significantly higher in niacin than all other hybrids used in this study.   In comparison of Ohio W36 with Ohio C38 and Ohio K24, the only difference in their genetic makeup is a single inbred; Ohio W36 having three high niacin inbred lines while Ohio C38 and Ohio K24 have only two high niacin inbred lines.   Hybrid Ohio M20 has four inbred lines

TABLE IV

VARIANCES FOR NIACIN AND PANTOTHENIC ACID CONTENTS
OF CORN HYBRIDS

Source of variance	Degrees of freedom	Niacin	Pantothenic acid
Location	6	17.76**	3.05**
Hybrid	7	21.77**	2.02**
Year	1	5.45*	2.49*
Location × hybrid	42	2.12*	.45
Location × year	6	11.29**	11.30**
Hybrid × year	7	4.15**	.87
Error	42	1.06	0.39
Total	111		

* Significant.   ** Highly significant.

of low niacin content in its genetic makeup and it was the lowest in niacin content of any one of the corn hybrids studied, with the possible exception of U.S. 379; while Ohio M34 has only three inbred lines of low niacin content and it was significantly higher in niacin than Ohio M20.   In a similar way, it can be shown why Ohio C38 and Ohio M34 have a significantly higher niacin content than U.S. 13 and possibly U.S. 379.   Unfortunately, the inbreds of which the corn hybrids are composed were not grown the same year or years, nor in the same location as the hybrids under study, and as a consequence the results (genetically speaking) may not appear to present as clear a relationship as might be expected.   This is no doubt due to difference in the environmental factor or factors to which the hybrids and inbreds were

[3] Lorraine Rodriguez, Chas. H. Hunt, and R. M. Bethke.   Protein, niacin, and pantothenic acid in corn inbred lines.   In print.

TABLE V

EFFECT OF LOCATION ON THE NIACIN CONTENT OF CORN HYBRIDS

Mahoning County, higher niacin content than Madison,* Belmont,* Miami,** Wayne,** and Hamilton.**

Paulding County, higher niacin content than Miami,** Wayne,** and Hamilton.**

Madison County, higher niacin content than Wayne** and Belmont.**

Belmont County, higher niacin content than Miami,* Wayne,** and Hamilton.**

Miami County, higher niacin content than Hamilton.**

* Significant.   ** Highly significant.

exposed.   However, it is interesting to note that the decreasing order of the niacin values of the corn hybrids is in the same position, with one exception, as the calculated values (average niacin content of inbreds for each hybrid).   See Tables I and III.

As previously stated, there was considerable variation in the niacin content of each hybrid from location to location.   The variances due to location, hybrid, year, and the various interactions are shown in Table IV.   The results of the analysis of variance due to location are shown in Table V.

A statistical analysis of the data (Table II) shows that Ohio W36 and Ohio M34 are significantly higher in pantothenic acid than Ohio C38, Ohio M20, Ohio C12, U.S. 379, and U.S. 13, while Ohio K24 is higher than U.S. 379 and U.S. 13.   Also, Ohio C38 is higher in pantothenic acid than U.S. 13.   Furthermore, Ohio M34 is significantly higher in pantothenic acid than Ohio M20.   Both have the same genetic parents, with one exception.   Ohio M34 has as one parent inbred (Oh 02), with a pantothenic acid content of 6.2 μg. per g., while Ohio M20 has as one parent inbred (Oh 33), with a pantothenic acid content of 4.1 μg. per g.   This clearly demonstrates the genetic influence on the hybrids themselves.   This is again substantiated by the fact that the decreasing order of the pantothenic acid values of the corn hybrids under study is, with two exceptions, in the same position as the calculated values (see Tables II and III).

TABLE VI

EFFECT OF LOCATION ON THE PANTOTHENIC ACID CONTENT
OF CORN HYBRIDS

Mahoning County, higher pantothenic acid content than Paulding,* Wayne,** Hamilton,** Madison,** and Miami.**

Belmont County, higher pantothenic acid content than Paulding,* Wayne,** Hamilton,** Madison,** and Miami.**

Paulding County, higher pantothenic acid content than Hamilton,* Madison,* and Miami.**

Wayne County, higher pantothenic content than Miami.*

* Significant.   ** Highly significant.

The calculated values as one observes are higher than the actual values.   This apparent discrepancy may be due to the difference in the environmental factors and the fact that the inbreds and hybrids were not taken from the same location and in the same year.

The results of the analysis of variance due to location are shown in Table VI.

The survey shows that corn hybrid Ohio W36 has the highest niacin content and, with one exception, the highest pantothenic acid content, while Ohio M20, U.S. 13, and U.S. 379 are the lowest in niacin, and U.S. 13 is the lowest in pantothenic acid.

The location effect shows that Mahoning County produced corn of the highest niacin and pantothenic acid content, while Hamilton County grew corn of the lowest niacin content, and Miami County, the lowest pantothenic acid content.

Acknowledgments

Literature Cited

HUNT, CHAS. H., DITZLER, LORRAINE, and BETHKE, R. M.   Niacin and pantothenic acid content of corn hybrids.   Cereal Chem. 24: 355–363 (1947).
DITZLER, LORRAINE, HUNT, CHAS. H., and BETHKE, R. M.   Effect of heredity on the niacin and pantothenic acid content of corn.   Cereal Chem. 25: 273–279 (1948).

THE EFFECT OF MATURITY ON THE NIACIN AND PANTOTHENIC ACID CONTENT OF THE STALKS AND LEAVES, TASSELS, AND GRAIN OF FOUR SWEET CORN VARIETIES[1]

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ABSTRACT

Niacin and pantothenic acid and percent dry matter were determined in the stalks and leaves, tassels, and grain of four varieties of sweet corn sampled at various stages of maturity. The dry matter and niacin content of stalks and leaves remained remarkably constant for the first six periods of collection or until the ears were well filled. The pantothenic acid content varied irregularly.

The niacin content of the tassels decreased regularly after pollination, with the highest value at that time. The pantothenic acid content of the tassel decreased as the plant matured but the decrease was not so regular as that of niacin. The niacin content of the pollen collected at bloom stage was higher than that of the tassel.

The dry matter of the grain increased two-fold over the period of collection and at the same time the niacin and pantothenic acid content decreased as the grain matured, but not proportionately to the increase in dry matter. The highest concentration of niacin and pantothenic acid in the grain occurred 18 to 20 days after the tassel bloomed. This is the "roasting-ear stage" when the corn should be consumed.

The possibility of developing high-niacin corn by breeding has been shown by several investigators (1, 2, 3, 4, 5, 6, 7). Hunt et al. (1947) and Ditzler et al. (1948) have also shown that pantothenic acid is regulated by inheritance and that the niacin and pantothenic acid contents of corn hybrids are influenced to some extent by such environmental factors as season and location.

The present investigations were undertaken to obtain information on the niacin and pantothenic acid content of the grain and other parts of the sweet corn plant at various stages of maturity, and to show the relation between the vitamin contents of the different parts of the plant. Earlier studies had shown that maturity affected the niacin and pantothenic acid content of field corn (unpublished data).

Materials and Methods

The varieties of sweet corn used were Marcross, Ohiogold, Golden Cross Bantam, and Stowell's Evergreen; all grown in the same field at

Wooster in 1948.   Marcross is an early-maturing variety, Ohiogold and Golden Cross Bantam are intermediate, and Stowell's Evergreen is a late-maturing variety.   Five replications of each variety were grown under similar environmental conditions.

The sampling procedure in the field was as follows: Samples were collected at seven to eleven-day intervals from July 19 to September 14, and again on October 21.   The stalks and leaves were collected throughout the period, tassels as soon as they appeared, and grain as soon as it was developed sufficiently for sampling.   One pollen sample from each variety was collected when the tassels were in full or near full bloom.

The stalks were cut at the first or second node above the ear, or, at the early dates, at the place where the ear was appearing.   This was partly determined by the maturity of the plant on the sampling date. The stalks and leaves were gathered as one sample, and the tassels and grain as separate samples.   Duplicate samples of leaves and stalks, tassels, and grain were gathered which were composites of the five replications.   These are designated as samples A and B.

Immediately after gathering, the stalk and leaves and tassel samples were chopped in a Hobart mill to form a mixture, after which a 100 g. portion was removed for moisture determinations.   The remaining part of the sample was run through a Waring Blendor and stored in a paraffined carton at freezing temperature (15°F.) until assayed.

The grains were cut from the cob and the cob was scraped to remove all of the grain.   The grain was then mixed in a Waring Blendor, a portion removed for moisture determination, and the remainder stored in paraffined cartons at freezing temperature.

The fresh or frozen samples were assayed microbiologically for niacin and pantothenic acid, according to methods described in a previous study (Hunt *et al.*, 1947).   The assay values were calculated to a dry matter basis.

Results and Discussion

The niacin and pantothenic acid contents as well as the percentage dry matter of the various parts of the sweet corn plant, at different stages of maturity, are shown in Table 1.   The data given are averages of samples A and B.

Marcross was the most mature of the four varieties at the date of the first collection, and it matured more rapidly than the other three varieties.   For this reason, samples collected at seven to eleven day intervals made a less complete record of the variation due to maturity in Marcross than in the other varieties.

The dry matter of the stalks and leaves of Marcross increased from 15.0 to 20.5% from July 19 to August 23; the niacin content increased,

irregularly, from 44.0 to 54.0 μg., and the pantothenic acid content increased from 11.7 to 18.9 μg. per g. in the same period.   The dry matter of the tassels of Marcross increased from 29.2 to 42.0% between July 19 and August 3, then decreased to 37.3% by August 23; while the niacin content of the tassels fell from 45.0 to 33.3 μg. between July 19 and July 27 and remained more or less constant thereafter. The pantothenic acid content of the same variety showed similar behavior, making its greatest decrease (37.1 to 23.0 μg.) from July 19 to July 27.   The dry matter of the grain of Marcross increased from 18.1 to 36.2%, a two-fold increase, from August 3 to August 23.   The niacin content decreased from 61.7 to 51.5 μg. from August 3 to August 23, while the pantothenic acid content showed little variation for the same period.

The variation in the niacin and pantothenic acid content of maturing Golden Cross Bantam and Ohiogold were generally similar. Stowell's Evergreen, a late-maturing variety, exhibited the same variation a few days later than the other two.

The dry matter of stalks and leaves of all three varieties (Golden Cross Bantam, Ohiogold, and Stowell's Evergreen) increased (15.3 to 26.2, 17.0 to 27.5, and 16.4 to 26.2%, respectively) from July 19 to September 14.

The niacin content of stalks and leaves of Golden Cross Bantam, Ohiogold, and Stowell's Evergreen remained relatively constant until the last two collection dates (September 7 and 14), when there was a marked drop.   The pantothenic acid content of the stalks and leaves of all three varieties (Golden Cross Bantam, Ohiogold, and Stowell's Evergreen) varied irregularly, being lowest at the middle collection period, with the highest values at the beginning and end of the collections.

The dry matter of the tassels of all three varieties (Golden Cross Bantam, Ohiogold, and Stowell's Evergreen) increased considerably (22.0 to 60.6, 24.2 to 61.4, and 21.0 to 58.9%, respectively).   The niacin content of the tassels of each corn decreased regularly, with the highest values at the first or second collection periods.   The pantothenic acid content of the tassels varied irregularly, with a general downward trend.

The dry matter of the grain of all three varieties (Golden Cross Bantam, Ohiogold, and Stowell's Evergreen) showed a two-fold increase over the period of collection (22.2 to 46.4, 18.0 to 41.8, and 16.6 to 33.6%, respectively).   The niacin and pantothenic acid contents of the grain decreased as the grain matured but not proportionately to the increase in dry matter.   Variations existed among the varieties as to the amounts of both vitamins present at any given stage of maturity.

TABLE I

EFFECT OF MATURITY ON THE NIACIN AND PANTOTHENIC ACID CONTENT
OF THE VARIOUS PARTS OF THE CORN PLANT.
MICROGRAMS PER GRAM. MOISTURE FREE

Date of harvest	Stalks and leaves			Tassels			Grain			Pollen	
	Dry matter %	Niacin	Panto-thenic acid	Dry matter %	Niacin	Panto-thenic acid	Dry matter %	Niacin	Panto-thenic acid	Niacin	Panto-thenic acid
MARCROSS											
Jul. 19	15.0	44.0	11.7	29.2	45.0	37.0				July 24	
Jul. 27	18.9	52.7	10.5	36.8	33.0	23.0					
Aug. 3	18.9	53.7	13.5	42.0	31.7	24.0	18.1	61.7	17.0	65.9	13.0
Aug. 12	20.5	46.8	14.8	39.6	27.8	23.5	23.9	61.9	20.4		
Aug. 23	19.1	54.0	18.9	37.3	32.0	21.8	36.2	51.5	16.6		
Oct. 21		9.7						26.0	8.0		
GOLDEN CROSS BANTAM											
Jul. 19	15.3	41.0	13.0								
Jul. 27	16.9	43.9	10.0	22.0	53.4	28.4					
Aug. 3	17.2	43.0	7.0	32.2	59.5	30.0					
Aug. 12	19.4	40.8	9.9	36.3	39.0	29.7					
Aug. 23	20.5	45.4	11.0	35.2	38.6	17.0	22.2	81.4	37.5	69.0	
Aug. 31	22.4	44.0	17.9	41.6	42.0	33.0	34.0	47.6	25.5		
Sept. 7	24.5	34.7	11.5	57.0	27.7	18.4	38.0	40.4	17.0		
Sept. 14	26.2	34.0	23.0	60.6	24.0	12.7	46.4	34.1	12.5		
Oct. 21		13.7						23.3	7.0		
OHIOGOLD											
Jul. 19	17.0	39.7	13.0								
Jul. 27	17.4	48.0	11.0	24.2	49.4	23.0					
Aug. 3	19.1	45.5	6.0	35.6	52.8	27.6					
Aug. 12	20.6	42.0	8.5	36.0	46.7	27.5					
Aug. 23	23.0	40.7	9.8	38.2	35.0	18.0	18.0	94.1	40.0	81.5	7.6
Aug. 31	23.2	44.6	13.8	41.6	37.6	28.0	30.6	59.2	30.6		
Sept. 7	26.5	34.0	7.0	52.0	31.5	18.0	34.3	60.6	17.0		
Sept. 14	27.5	36.7	18.5	61.4	25.0	19.7	41.8	49.3	13.0		
Oct. 21		16.0						37.0	7.5		
STOWELL'S EVERGREEN											
Jul. 19	16.4	42.0	12.0								
Jul. 27	17.4	46.9	10.0	21.0	58.8	26.0					
Aug. 3	18.6	51.0	6.6	32.8	53.7	23.6					
Aug. 12	19.5	44.4	9.6	32.8	46.0	24.9					
Aug. 23	23.3	37.8	8.0	37.6	38.7	13.0	16.6	80.8	30.0	56.0	13.5
Aug. 31	22.9	41.8	9.6	45.2	39.0	22.0	24.1	94.4	27.4		
Sept. 7	26.0	35.8	7.6	52.0	30.0	12.0	27.8	80.4	19.6		
Sept. 14	26.2	36.6	12.0	58.9	28.7	18.0	-33.6	62.5	18.0		
Oct. 21		13.0						48.7	3.0		

The vitamin contents of both stalks and leaves and grain of all varieties collected on October 21 demonstrate that the downward trend continued after the regular collections were stopped. The varietal variations evident earlier in the vitamin contents of grain continued, and varietal variations in vitamin content of dry stalk and leaf tissue became evident.

As noted earlier, the vitamin content of the stalks and leaves of all varieties remained relatively constant until the ear was well formed. This may be attributable to the fact that the primary function of the leaf is to synthesize carbohydrates, which are then translocated to the ear. It may be that a similar synthesis and translocation can be said to occur in the case of niacin and pantothenic acid.

In the tassels, the highest concentration of the vitamins, particularly niacin, occurred just before or during the dispersion of the pollen "bloom stage." The niacin content of the pollen was higher than that of the tassel at this stage. The same was not true of pantothenic acid.

The highest concentration of both vitamins in the grain occurred eighteen to twenty days after the "bloom" stage, or when the pollen was dispersed. Corn consumed at this stage is succulent and sweet and is capable of adding considerable niacin and pantothenic acid to the human dietary.

Acknowledgments

Literature Cited

1. BARTON-WRIGHT, E. C., Microbiological assay of nicotinic acid. Biochem. J. 38: 314–319 (1944).
2. BURKHOLDER, P. R., McVEIGH, ILDA, and MOYER, DOROTHY. Niacin in maize. Yale J. Biol. Med. 16: 659–663 (1944).
3. DITZLER, LORRAINE, HUNT, CHAS. H., and BETHKE, R. M. The effect of heredity on the niacin and pantothenic acid content of corn. Cereal Chem. 25: 273–279 (1948).
4. GORFINKEL, L. The influence of crossing on the nicotinic acid content of maize. Jour. Agr. Sci. 38: 339–342 (1948).
5. HUNT, CHAS. H., DITZLER, LORRAINE, and BETHKE, R. M. Niacin and pantothenic acid content of corn hybrids. Cereal Chem. 24: 355–363, (1947).
6. MATHER, K., and BARTON-WRIGHT, E. C. Nicotinic acid in sugary and starchy maize. Nature 157: 109–110 (1946).
7. RICHEY, FREDERICK D., and DAWSON, R. F. A survey of the possibilities and methods of breeding high niacin corn (maize). Plant Phys. 23: 238–254 (1948).

THE QUALITY OF HARD RED WINTER WHEAT AS AFFECTED BY 2,4-D SPRAY APPLICATIONS [1,2]

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ABSTRACT

Samples of hard red winter wheat grown in 1948 at the Fort Hays (Kansas) Branch Experiment Station and representing various dates, types, and dosages of 2,4-D application revealed no reduction in wheat yield resulting from the treatment with 2,4-D. Only one date of application of 2,4-D in four produced a significant increase in wheat protein. When yield of protein (per cent protein × yield) was considered there was no difference between treated and untreated samples. Applications of 2,4-D did not affect the mineral content, milling, or baking quality of the wheats. These conclusions were based on one year's samples, obtained from one location.

Erickson, Seely, and Klages (2) and Sibbitt and Harris (7) have reported that certain applications of 2,4-D treatments on weed-free plots increased the protein content of the kernels. Sibbitt and Harris (7) have also reported that applications of the acetate, the triethyl amine, and the butyl ester of 2,4-dichlorophenoxyacetic acid to wheat plants at different stages of growth did not impair the milling or baking quality of the wheat. Several varieties of both spring and winter wheat were investigated (2, 7) and at the Idaho Experiment Station (2) the trials were conducted on both dry land and irrigated plots.

Klingman (4) and McNeal (5) have shown that wheat yields are usually reduced significantly by 2,4-D treatments. Since previous workers have not reported on the protein content of wheat as related to yield, it seemed desirable to study the effect of 2,4-D treatments on the protein content of wheat when yield of protein (protein × yield) is considered. The effect of 2,4-D treatments on the mineral content of wheat was also investigated.

Materials and Methods

The wheat for this study was grown at the Fort Hays Branch Experiment Station in Central Kansas and harvested in 1948. The wheat was normal as indicated by many comparative analyses and milling and baking tests (6).

The date and type of treatment on the randomized plots, replicated three times, are shown in Table I.   Methods of application included both ground and airplane spraying; however, in the interest of shortening and simplifying this presentation, only the ground spraying experiments are included.   The results were essentially the same in both experiments.   One hundred and thirty-five samples representing various dates, types, and dosages of 2,4-D applications were harvested. All the wheat plots, including the check plots, were hand-weeded. Moisture, protein, and ash determinations were made on all samples using the methods outlined in *Cereal Laboratory Methods*, 5th ed. (1).

The baking tests were conducted using a standard sponge formula and baking procedure as described by Johnson and Miller (3).   A total of 700 g. of flour was mixed in a Hobart A-200 mixer.   Two 250-g. doughs were divided and baked from each mix.

Results and Discussion

Table I summarizes the yield, protein, and ash determinations for the wheat samples subjected to various 2,4-D treatments at different stages of growth.

A summary of the analysis of variance for ash, protein, and protein $\times$ yield data is presented in Table II.   Variation between groups was significant for ash, protein, and yield, but not for yield of protein.   Replications within groups were also significant for all of the factors studied.   This can possibly be explained by the wide distribution of the plots, although the entire experiment was conducted in the same area on what appeared to be uniform soil conditions.

Yield.   The various 2,4-D treatments did not reduce the yield of wheat significantly (Table II).   This is not in agreement with the work of Klingman (4) or McNeal (5).   However, the evidence presented here indicates that the trend in treated plots as compared with untreated plots is toward a reduction in yield of wheat.

Protein.   Examination of Table I shows that there is no consistent change in protein content with increased dosages of the various treatments.   In most instances, however, the protein content of the wheat tended to increase by 2,4-D applications as compared to the untreated plots.   Group C was the only group which showed significant treatment differences with respect to per cent protein.   The grain in this group was in the heading stage during the application of the spray. Rainfall following this application amounted to 4.27 in. during the next 14 days and temperatures were relatively low during this period. In contrast only 1.23 in. of rain were received in the two weeks following the April 23 treatment and none from May 11 to May 29.

TABLE I

EFFECT OF VARIOUS 2,4-D SURFACE SPRAY APPLICATIONS ON THE YIELD,
PROTEIN, AND ASH OF HARD RED WINTER WHEAT

Formulation	Mean values		
	Yield	Protein[1]	Ash[1]
	bu./A.	%	%
GROUP A			
Date of treatment—April 26, 1949; stage of growth—pre-fully tillered			
None	50.8	14.6	1.69
Sodium salt[2]	50.0	14.5	1.75
Butyl ester[3]	45.5	14.7	1.71
Butyl ester+diesel oil[4]	50.1	14.5	1.73
Average difference due to treatment	− 2.3	0.0	+0.04
GROUP B			
Date of treatment—May 11, 1949; stage of growth—second joint			
None	47.6	14.5	1.71
Sodium salt[5]	48.0	14.8	1.73
Butyl ester[6]	44.5	14.7	1.78
Butyl ester+diesel oil[7]	46.0	14.6	1.81
Average difference due to treatment	− 1.4	+ 0.2	+0.06
GROUP C			
Date of treatment—May 27, 1949; stage of growth—early heading			
None	41.1	14.7	1.63
Sodium salt[2]	43.0	14.7	1.69
Butyl ester[3]	39.0	15.2	1.68
Butyl ester+diesel oil[4]	38.5	15.1	1.68
Average difference due to treatment	− 0.9	+ 0.3	+0.05
GROUP D			
Date of treatment—June 23, 1949; stage of growth—late dough			
None	44.0	14.5	1.66
Sodium salt[2]	42.3	14.8	1.66
Butyl ester[3]	45.0	14.6	1:66
Butyl ester+diesel oil[4]	44.2	14.8	1.62
Average difference due to treatment	− 0.2	+ 0.2	−0.01

[1] Results reported on 14% moisture basis.
[2] Mean of results from triplicated plots to which 0.25, 0.5, and 1.0 lb. quantities of the sodium salt of 2,4-dichlorophenoxyacetic acid were added as a surface spray.
[3] Same as footnote 2, except the butyl ester of 2,4-dichlorophenoxyacetic acid was employed.
[4] Same as footnote 2, except the butyl ester of 2,4-dichlorophenoxyacetic acid in 5 qts. of diesel oil was employed.
[5] Same as footnote 2, except 0.25, 0.5, 1.0, and 1.5 lb. quantities of the sodium salt of 2,4-dichlorophenoxyacetic acid were employed.
[6] Same as footnote 2, except 0.12, 0.25, 0.5, 0.75, and 1.0 lb. quantities of the butyl ester of 2,4-dichlorophenoxyacetic acid were employed.
[7] Same as footnote 2, except 0.12, 0.25, 0.5, 0.75, and 1.0 lb. quantities of the butyl ester of 2,4-dichlorophenoxyacetic acid in 5 qts. of diesel oil were employed.

Average protein differences were sufficiently large to indicate a significant treatment effect in the analysis of the data from all groups (Table II). Least significant mean differences determined in the usual way showed that the mean value of per cent protein for Group C was significantly higher than that for any of the other groups. However, when total protein (per cent protein × yield) data were

TABLE II

EFFECT OF 2,4-D TREATMENTS ON WHEAT YIELD, PROTEIN, ASH, AND YIELD OF PROTEIN (PROTEIN × YIELD)

Sources of variation	Degrees of freedom	Mean squares yield	Mean squares protein	Mean squares ash	Mean squares protein × yield
ANALYSES OF VARIANCE BY GROUPS					
Group A—Treatment April 26, 1948—Stage of growth, pre-fully tillered					
Replications	2	143.4**	0.36**	0.009	2.18*
Treatments	9	19.6	0.02	0.003	0.33
Error	17	23.4	0.04	0.003	0.48
Total	28				
Group B—Treatment May 11, 1948—Stage of growth, second joint					
Replications	2	321.7**	0.04	0.063*	6.50**
Treatments	14	39.0	0.10	0.018	0.76
Error	28	37.7	0.17	0.018	0.81
Total	44				
Group C—Treatment May 27, 1948—Stage of growth, early heading					
Replications	2	154.7**	0.34**	0.018	2.56**
Treatments	9	16.7	0.23**	0.005	0.22
Error	18	14.2	0.05	0.006	0.32
Total	29				
Group D—Treatment June 23, 1948—Stage of growth, late dough					
Replications	2	148.8**	0.34**	0.013**	2.58**
Treatments	9	12.4	0.06	0.002	0.26
Error	18	18.9	0.05	0.001	0.41
Total	29				
ANALYSES OF VARIANCE FOR ALL GROUPS					
Groups	3	418.0***	0.92**	0.079*	6.86
Treatments	9	27.7	0.18*	0.004	0.44
Replications within groups	8	142.1***	0.26**	0.014*	2.48**
Groups × treatments	27	16.3	0.09	0.005	0.30
Error	71	25.0	0.08	0.005	0.54
Total	118				

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

analyzed, the treatment differences for this group were no longer significant.

Ash. The statistical calculations (Table II) revealed no significant differences in the mineral content of the wheat as a result of 2,4-D applications. Differences due to replications, however, were significant within certain groups.

Yield of Protein (Yield × Protein). Several workers (2, 7) have reported that 2,4-D treatments increased the protein content of wheat. For practical purposes, "yield of protein" representing both yield and protein content should also be considered, since it is recognized generally that the protein content of wheat is higher when grown under conditions causing reduced yields. It seems probable that the reported protein increase caused by 2,4-D applications was obtained at a sacrifice in yield. In the reports previously published (2, 7) protein content of 2,4-D treated plots have not been analyzed statistically nor discussed in relation to wheat yield data. However, in this work based on one year's study at one location, 2,4-D applications did not produce a significant difference in "yield" of protein.

Milling and Baking Tests. There was no indication that the milling quality of wheat was influenced by the spray treatments. Similarly the baking quality of the treated wheats was not impaired. This corroborates the work of Sibbitt and Harris (7). Samples in Group A (Table I) showed a slight increase in loaf volume with increasing concentrations of all three types of spray. Application of sprays at later stages of development did not produce similar effects, although the wheats produced were somewhat superior to those in Group A with respect to both protein content and loaf volume.

Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal Laboratory Methods, 5th ed. Am. Assoc. Cereal Chem. University Farm, St. Paul 1, Minn. (1947).
2. ERICKSON, LAMBERT C., SEELY, C. I., and KLAGES, K. H. Effect of 2,4-D upon the protein content of wheats. Jour. Am. Soc. Agron. **40**: 659–660 (1948).
3. JOHNSON, JOHN A., and MILLER, BYRON S. Studies on the role of α-amylase and proteinase in breadmaking. Cereal Chem. **26**: 371–383 (1949).
4. KLINGMAN, DAYTON L. Effects of spraying cereals with 2,4-dichlorophenoxy-acetic acid. Jour. Am. Soc. Agron. **39**: 445–447 (1947).
5. McNEAL, F. H. Effect of 2,4-D on the yield and height of Federation wheat. Jour. Am. Soc. Agron. **40**: 1070–1073 (1948).
6. SHELLENBERGER, J. A., FINNEY, K. F., MILLER, B. S., McCAMMON, J. F., LEWIS, E. M., and JOHNSON, J. A. Twelfth annual report of the Hard Wheat Quality Laboratory. 1948. U. S. Dept. of Agriculture, Bureau of Plant Industry, Soils and Agricultural Engineering, Division of Cereal Crops and Diseases.
7. SIBBITT, L. D., and HARRIS, R. H. A note on the effect of chlorine-substituted phenoxy compounds on the milling and baking quality of hard red spring wheat. Cereal Chem. **25**: 286–288 (1948).

PREPARATION OF DRIED NATIVE WHEAT GLUTEN [1]

C. V. Lusena

ABSTRACT

A new method of preparing dried purified wheat gluten that retains its
native properties consists of dispersing a washed gluten in 0.005 N acetic
acid, centrifuging, and vac-ice drying the dispersion (lyophilizing). Washed
gluten was used because the salts present in flour substantially reduced
the solubility of gluten protein at concentrations of about 0.5 g. per liter of
solution. Centrifuging removed the starch quantitatively. The dried
gluten is a stable white powder containing 8% bound crude fat. Baking
tests show that the purified gluten was substantially undamaged.

The preparation of suitable bulk samples of wheat gluten for
research purposes is difficult and the usual practice has been to start
each test with a separate portion of flour and either disperse it in a
gluten solvent or use it to prepare a washed gluten for dispersion.
This individual treatment of samples is not ideal because it may lead
to considerable variation both in the purity of the gluten and in the
extent to which it has been altered within a single series of tests.

The object of the present investigation was to prepare bulk samples
of gluten for experimental purposes. Obviously, to be of value, the
prepared gluten should be as near to its native state as possible, and
it should be purified, stable, and easily handled. These requirements
suggested that gluten should be dispersed in a mild solvent, purified,
recovered from dispersion, dried, and, if necessary, ground.

Dilute acetic acid appeared to offer most promise as a gluten
solvent. Neutral solvents have been considered milder (3) but the
slow hydrolysis observed in acetic acid has been shown to be due to an
enzyme (10). Concentrations of acetic acid as low as 0.01 N have
been suggested (10). Strong acids at concentrations as low as 0.1 N
have been used but they appear to be less satisfactory than weak acids
(6). Recovery of gluten from dilute acetic acid dispersions demands
only that the acetic acid be neutralized (11).

The literature is less informative on the best method of preparing
gluten dispersions. The relation between lipoids and gluten is com-
plex; ether will extract fats from normal flour but not from well
moistened flour (9). Dispersions have been prepared in dilute acetic
acid directly from flour (6), but other investigators have been unable

to use flour directly (11).   Salts, even at low concentrations, affect the
solubility of gluten in acids (14).   The more common practice is to
prepare crude gluten by making flour into a dough and then washing
it in tap water or buffer solution.   The resulting mass requires either
a long contact time with the solvent or mechanical treatment.   The
use of the latter has been criticized on the basis that it changed the
shape of the ultracentrifuge sedimentation diagrams (7), but as the
treatment given involved precipitation and redispersion, the evidence
against mechanical treatment is not conclusive.   In view of the ap-
parent contradictions in the literature, preparations of dispersions from
flour and from washed gluten were compared.

Gluten dispersions and in some cases recovered gluten were dried
by freezing the mixture and removing the moisture under vacuum.
This method, sometimes called lyophilization, is referred to in this
paper as vac-ice drying.

The final step was to determine if the purified gluten had retained
its native characteristics.   Probably the most comprehensive test of
gluten quality is the baking test; it has been used to evaluate the
quality of crude gluten (1, 4, 5).   Consequently, in this study, the
gluten preparations were subjected to baking tests.   A recommended
procedure of preparing purified gluten is given at the end of this paper.

Materials and Methods

The flour was a commercial first patent flour containing 12.8%
protein (14% moisture basis), prepared from Western Canadian
Spring Wheat No. 1 and No. 2 Northern.   The manufacturer revealed
that it had been aged with nitrogen trichloride (Agene) and bleached
with benzoyl peroxide (Novadelox).   As most investigators have used
unmodified (non-extracted) flour the fats were not extracted from the
flour in this study.

Nitrogen contents were determined by micro or macro Kjeldahl
methods.   Moisture content was determined by drying to constant
weight at 75°C. at less than 1 mm. of mercury pressure, ash by
ignition at 600°C., and starch polarimetrically (2).   Crude fat was
determined as follows: the samples were extracted with absolute
ethanol for 18 hr. in a Soxhlet extractor, the extract was evaporated
to dryness at 100°C., the residue was extracted with chloroform, the
chloroform extract was filtered and dried at 65°C. overnight and
weighed as crude fat.   Hydrogen ion concentration was determined
electrometrically using a glass electrode.   Concentrated gluten dis-
persions were diluted to approximately 1 mg. of nitrogen per ml. of
dispersion prior to determining the pH; otherwise the protein co-
agulated on the electrodes.   Diluting dispersions containing 10 mg.

of nitrogen per ml. had no effect on the pH value obtained, so it was assumed that diluting more concentrated dispersions did not alter the pH appreciably.

The baking formulas employed by Aitken & Geddes (1) were used.

Results

Choice of Material for Dispersion. Preliminary tests showed that crude gluten would disperse almost quantitatively in 0.01 N acetic acid to give dispersions containing 8 mg. of nitrogen per ml. of dispersion (4.5% gluten) but when flour containing the amount of gluten needed to give a dispersion of this concentration was used, only a small fraction of the gluten protein dispersed. The cause of this was traced to a water-soluble material since making flour into a dough did not increase the amount of protein which dispersed in acetic acid, whereas stirring flour in water, centrifuging, and discarding the liquid increased the amount of gluten that dispersed in acetic acid to the same value as when crude gluten was used.

The effect of water-soluble flour components on the solubility of gluten in 0.01 N acetic acid was investigated further. The material precipitated by 70% alcohol from an aqueous extract of flour had no effect on the solubility of gluten in 0.01 N acetic acid, nor did the material left after dialysing an aqueous extract of flour. Various 0.01 N acetic acid solutions containing different amounts of ash were prepared by adding aqueous extract of flour, the 70% alcohol soluble fraction of an aqueous extract, the dialysate of an aqueous extract, the ash from an aqueous extract of flour, and salts to simulate a synthetic ash.[2] Then 5.0 g. portions of vac-ice dried gluten (prepared by a procedure given later) were dispersed in 100 ml. portions of the various solutions with a Waring Blendor. The vac-ice dried gluten dispersed in aqueous acetic acid in less than 2 min. and was not affected by prolonged stirring; a 5 min. mixing time was allowed with the acetic acid solutions containing ash so that a slower rate of dispersion would not be confused with insolubility. The results in Fig. 1 show that, irrespective of the source of ash, a concentration of 0.5 to 0.75 g. per liter was sufficient to reduce substantially the solubility of gluten. The pH of the dispersions did not vary enough to have any effect on the amount of gluten dispersed.

These results show the necessity of removing the soluble ash from flour prior to dispersing the gluten. Starch does not decrease the solubility of gluten but it increases the amount of the dispersion which is lost during purification. Consequently, crude gluten was selected as the best starting material for gluten dispersions.

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[2] One liter of salt solution contained 1.67 g. of KH_2PO_4, 0.47 g. of $Mg_3(PO_4)_2 \cdot 4H_2O$, 0.31 g. of Na_2SO_4, 0.23 g. of $MgSO_4$, 0.17 g. of $Ca\ SO_4$, 0.12 g. of $MgCl_2$ and 0.03 g. of $FeSO_4 \cdot 7H_2O$.

Dispersion of Crude Gluten. Crude gluten was dispersed in 0.01 and 0.005 N acetic acid with a Waring Blendor and at the end of the mixing time five drops of n-octyl alcohol were stirred in. Since the toughness of crude gluten is increased by a more complete removal of the starch and since the extent of mechanical treatment required is obviously related to the tenacity of the crude gluten, tests were undertaken to establish the best washing conditions. Three samples of wet gluten were prepared that contained, on analysis, 42%, 25%, and 6% starch respectively, on a dry basis. Portions of each, sufficient to

ASH CONTENT OF SOLVENTS - GM. PER LITRE
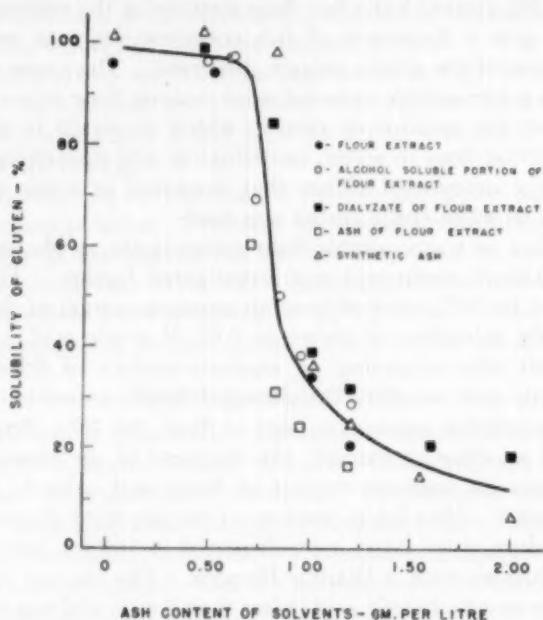
Fig. 1. Effect of salts on the solubility of gluten in 0.01 N acetic acid.

give approximately 4% gluten dispersions, were mixed with the solvents in a Waring Blendor for varying periods and the proportion dispersed was determined. The results, recorded in Table I, show that except for the toughest gluten (Sample 3), 3 min. stirring was sufficient. Large particles of Sample 3 were apparent at the end of this period, but after 7 min. stirring it gave the highest percentage dispersion. Tests showed that the lower percentage dispersion with the other samples was not due to salts, and therefore, the more complete dispersion of Sample 3 is attributed to a more thorough removal of acid-insoluble components during washing. Sample 1 also gave a higher loss of gluten during purification owing to the amount adhering to the starch, which was removed by centrifuging.

The gluten containing 25% starch was considered satisfactory and a stirring time of 5 min. was ample. The gluten washing procedure developed by Shewfelt and Adams (14) yields a gluten containing approximately 25% starch and was accordingly adopted. Details are given in the last section of this paper.

As 0.005 N acetic acid disperses gluten, it seemed worthwhile to determine if dispersion were a function of pH alone. Dispersions were prepared in 0.0025, 0.005, and 0.01 N hydrochloric acid. The first failed to disperse the same amount as 0.005 N acetic acid. The pH of a dispersion in 0.005 N hydrochloric acid containing 7.0 mg. of nitrogen per ml. of dispersion was 4.9 as compared to 5.1 in 0.005 N acetic acid. This similarity suggested that pH was the important factor.

Purification of Gluten Dispersions. Preliminary tests on dispersions containing 7 mg. nitrogen per ml. of dispersion showed that centrifug-

TABLE I

EFFECT OF STARCH CONTENT ON THE DISPERSABILITY
OF GLUTEN IN ACETIC ACID

Sample	Starch content. % dry matter	Protein dispersed in 0.005 N acetic acid, %		Protein dispersed in 0.01 N acetic acid, %		
		After 3 min. stirring	After 5 min. stirring	After 3 min. stirring	After 5 min. stirring	After 7 min. stirring
1	42	94.4	94.6	95.0	95.1	95.0
2	25	96.2	96.3	96.3	96.0	96.2
3	6	10	93.3	15	93.6	98.1

ing in bottles of 200 ml. capacity at 2,000 times gravity for 10 min. effectively sedimented undispersed material. As purification was probably related to viscosity, i.e., to gluten concentration, different dispersions were centrifuged at the same rate and for the same time and their starch contents determined. Cooling the dispersions during preparation increased the starch content of the purified gluten, therefore, extracts were made and centrifuged at room temperature. The results recorded in Table II show that the starch content, expressed as a percentage of the dry matter, remained almost constant up to a concentration of 7 mg. nitrogen per ml. but increased with concentration thereafter. Longer centrifuging would have purified more concentrated dispersions, but for convenience, 7 mg. nitrogen per ml. of dispersion was accepted as a suitable concentration. Purification by precipitation and dispersion is reported later.

Recovery of Gluten. Gluten is readily salted out from an acetic acid dispersion, but as this would contaminate the gluten with salt, it

TABLE II
EFFECT OF GLUTEN CONCENTRATION ON PURIFICATION

Concentration of acetic acid, N	Concentration of dispersion, mg. nitrogen per ml.	pH of dispersion	Starch content of gluten, %, dry matter basis
0.005	4.2	4.7	0.06
	5.5	4.9	0.06
	6.1	5.0	0.06
	6.7	5.0	0.06
	7.9	5.2	0.10
0.01	4.3	4.5	0.06
	5.6	4.6	0.06
	6.0	4.7	0.05
	6.7	4.8	0.06
	7.4	4.8	0.07
	8.1	4.8	0.09
	8.8	4.9	0.09
	9.3	4.9	0.08

was abandoned in favor of neutralizing the acetic acid with saturated calcium hydroxide solutions. When gluten dispersion was added to calcium hydroxide solution the pH of the supernatant liquid was lower than when the alkali was added to the dispersion. Above pH 9.5, gluten redispersed. To avoid high local concentrations, either a Waring Blendor or an ordinary stirrer was employed.

The relation between pH, as influenced by the addition of calcium hydroxide solution, and gluten recovery with each stirring method is shown in Fig. 2. At the lower pH values, recovery was less with the mechanical stirrer—probably because the protein had a lower pH than the liquid—but at pH 6.5, and higher, recovery was better with the stirrer; the Waring Blendor tended to redisperse the gluten as alkalinity increased. The mechanical stirrer also had the advantage
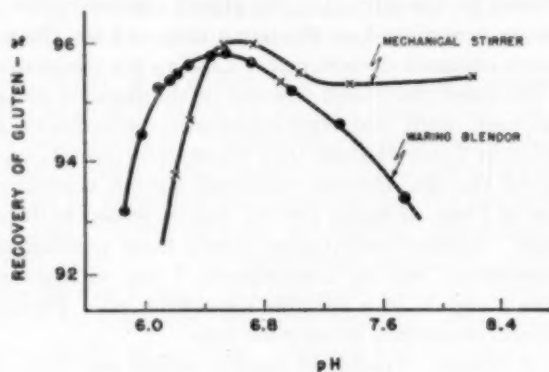
Fig. 2.   Effect of pH on the recovery of gluten.

that all but about 0.5% of the gluten collected on the stirrer shaft and
was easily removed.   (The remaining 0.5% can be centrifuged out and
collected if desired.)   The stirrer is therefore preferred during pre-
cipitation.   Pressing and draining the gluten made it more tenacious
and greatly interfered with redispersion.

Second Dispersion and Purification.   With a Waring Blendor, a
number of dispersions of soft (unpressed) gluten in 0.01 N acetic acid
were prepared from one sample of recovered gluten.   The amounts
used varied from 50 to 250 g. of wet gluten per 250 ml. of 0.1 N acetic
acid.   Over 99.5% of all the amounts up to and including 200 g.
dispersed.   As the gluten contained water, 200 g. would dilute the
acetic acid to about 0.006 N.   The dispersions were centrifuged and
the amount of sedimented starch determined.   The results given in
Table III show that the amount of starch sedimented decreased as the

TABLE III

DISPERSION AND PURIFICATION OF RECOVERED GLUTEN

Conc. of dispersion, mg. nitrogen per ml.	pH of dispersion	Gluten dispersed, %	Starch sedimented, % by weight of gluten
8.5	5.1	99.7	0.12
10.3	5.2	99.8	0.11
11.5	5.3	99.6	0.11
12.7	5.3	99.7	0.08
14.5	5.4	99.7	0.07
16.1	5.5	99.6	0.05
17.1	5.5	99.2	0.04
19.1	5.5	99.1	0.04
21.0	5.6	99.7	0.04
23.4	5.6	99.6	0.04
19.9	5.7	65.0	—

gluten concentration increased.   A concentration of 10–12 mg. nitro-
gen per ml. of dispersion permitted satisfactory purification and
further dilution had no effect on the amount of starch which was
sedimented.   The results also show that up to 23 mg. of gluten
nitrogen per ml. can be dispersed.   No gluten sedimented out during
10 days cold storage.

Repeated recovery and dispersion of gluten appeared to remove a
non-gluten nitrogenous substance; after the first recovery 4–5% of the
nitrogen remained in the supernatant liquid, but with repeated recovery
and dispersion this value fell to about 1% and the concentration of
nitrogen in the supernatant liquid dropped to, and remained at, 0.12–
0.14 mg. nitrogen per ml.   This probably represents the solubility of
gluten in the supernatant liquid.

Vac-ice Drying of Gluten.   Dispersions containing 8–23 mg. nitro-
gen per ml. were poured into aluminum trays to a depth of 0.5 cm. and

frozen at − 18°C. This required about 2 hr. The trays were placed in a vacuum chamber on heavy metal shelves and the pressure maintained below 1 mm. of mercury. To counteract cooling due to evaporation, heat was applied electrically to maintain the temperature of the shelves at − 1?C. The evaporated moisture was condensed on a coil which was held at − 20°C. Drying required 36 to 48 hr. The gluten remained as a white layer which showed the crystal pattern of ice. It was readily crumbled into a powder when passed through a 40-mesh sieve.

Properties of Vac-ice Dried Gluten. The vac-ice dried gluten readily formed a tenacious glutenous mass when mixed with water in the ratio of 1 g. of gluten to 1.5 ml. water. It dispersed quantitatively in distilled water up to concentrations of 17 mg. nitrogen per ml. (with a Waring Blendor) and the pH of the dispersions was below 5.0. This solubility and the low pH were attributed to bound acetic acid. Acetic acid evaporates readily under the conditions of vac-ice drying in the absence of gluten, but analyses showed that gluten dried from dispersions retained about 0.5% acetic acid. On neutralizing the gluten, by adding dilute sodium hydroxide, the solubility decreased to 0.14 mg. nitrogen per ml., i.e., to the solubility of recovered gluten in distilled water. The pH of minimum solubility was 6.55, which is the same as that of recovered gluten.

A typical analysis of a preparation follows:—

	Dry matter basis, %
Protein (nitrogen × 5.7)	90.0
Crude fat (alcohol soluble)	8.0
Ash	0.5 (or less)
Acetic acid	0.5
Starch	0.01 (or less)

Dry matter was normally above 92%. The crude fat in the gluten was not ether-soluble.

Baking Quality Tests. To determine the effect of varying the conditions of preparation of gluten, 12 samples were made. The pH of the first dispersion was varied from 4.8 to 5.2 by changing the concentration of acetic acid and the pH of the second dispersion, from 4.3 to 5.5. One sample was recovered and vac-ice dried, the others were vac-ice dried directly from dispersion.

A soft white spring wheat flour containing 8.0% protein (14% moisture basis) was used as the base flour for baking tests and gluten preparations were added to increase the protein to 13.5%. As a control, an air-dried crude gluten was prepared from the same flour by the method of Aitken and Geddes (1) as they have shown that this method gives an undamaged gluten. Preliminary trials showed that the malt-phosphate formula was more satisfactory than the malt-

phosphate-bromate formula as it gave loaf volumes 40 cc. higher with
the base flour, 45 cc. higher with the base flour plus control gluten,
and 80 cc. higher with the base flour plus purified gluten.   Examina-
tion of the physical appearance of the crust showed that the loaves
had a characteristic "over-aged" appearance.   The possibility was
tested that n-octyl alcohol (which was used as an antifoam) might
interfere but the amount that could be present (even if none were lost
during drying) had little effect on the volumes of loaves made from an
ordinary flour.

TABLE IV

EFFECT ON BAKING QUALITY OF INCREASING THE PROTEIN CONTENT OF A
SOFT WHEAT FLOUR TO 13.5% WITH VARIOUS GLUTEN PREPARATIONS

Gluten prepara- tion	Description of added gluten	pH, first disper- sion	pH, second disper- sion	Loaf volume, cc.	Crumb	
					Color	Texture
	None added	—	—	450	4.5	4.5
	Control gluten (air dried)	—	—	680	4.5	4.5
1	Recovered and dried	4.8	—	655	5.0	6.0
2	Dispersion dried	4.8	5.5	660	5.0	5.5
3	As 2, neutralized	4.8	5.5	665		
4	Dispersion dried (Stored 6 months at 5°C.)	5.0	5.2	695	6.0	6.0
5	Dispersion dried	5.2	5.5	705	5.0	6.0
6	Dispersion dried	4.8	5.6	640	5.0	5.5
7	Dispersion dried	4.8	5.2	620	5.0	5.5
8	Dispersion dried	4.8	5.1	630	5.0	5.5
9	Dispersion dried	4.8	4.9	615	5.0	5.5
10	Dispersion dried	4.8	4.7	600	4.5	5.5
11	Dispersion dried	4.8	4.5	565	4.0	5.5
12	Dispersion dried	4.8	4.3	540	4.0	5.5

Preparations 1–12 were vac-ice dried.
   All 1st dispersions were in 0.01 N acetic acid with the exception of Nos. 4 and 5 where 0.005 N
acetic acid was used.
   The 2nd dispersion for No. 2 to No. 6 were in 0.01 N acetic acid.   For the subsequent samples the
acid concentration was proportionately increased to 0.05 N.
   Baking results are means of duplicates.

As the malt-phosphate formula gave higher loaf volumes it was
used in the main series.   Duplicate loaves were baked and the mean
results are recorded in Table IV.   The baking results suggest that
the best gluten preparations, Nos. 4 and 5, were slightly better than
the control gluten.   Dried recovered gluten, No. 1, was not signifi-
cantly different from dried dispersed gluten, No. 2.   Neutralized
gluten, No. 3, was little better than No. 2.   Preparation No. 4, which
had been stored for six months at 5°C., was similar to a new sample,
No. 5.   Increasing acid concentration both in 1st and 2nd dispersions
affected the baking qualities of the gluten adversely.   For comparison,
a hard red spring wheat flour containing 13.5% protein was baked; the
loaf volumes were 715 cc. by the malt-phosphate formula and 815 cc.
by the malt-phosphate-bromate formula.

Discussion

The effect of salts on the dispersion of gluten in dilute acetic acid has been shown.   The critical concentration of salts which interferes with the dispersion of gluten is about 0.5 g. per liter.   If a flour containing 0.5 ash and 10% gluten is used directly, the soluble salts present will interfere with the preparation of dispersions containing more than 1.4% gluten, i.e., 2.5 mg. of nitrogen per ml. of dispersion. Below this concentration normal flour does not contain enough soluble salts to interfere.   This probably accounts for some of the inconsistencies in the literature.   It is significant that those who have used flour directly have prepared only dilute gluten dispersions.

In the preparation of purified gluten of good baking quality the pH of the dispersion is important.   That of the first dispersion should be 5.0 or higher and that of the second 5.5 or higher.   Neither the dispersion of gluten nor its recovery reached 100%; the former is attributed not to the presence of salts but to an acid-insoluble material that is removed on washing, and the latter to a water soluble nongluten fraction that has been recognized by others (8, 13) and also to the slight solubility of gluten.

The loaf volume for a "strong" flour by the malt-phosphate formula was only 10 cc. higher than that of a flour fortified with the best gluten (No. 5, Table IV).   The addition of bromate increases the loaf volume of a strong flour by 100 cc., but it decreased the loaf volume of a vac-ice gluten fortified flour by 80 cc. and that of a control gluten fortified flour by 45 cc.   These results and the "over-aged" appearance of the crust are partly due to "aged" flour being used to prepare the gluten.   Some difference in bromate response between the "fortified" flour and that of a "strong" wheat flour would be expected because part of the protein of the former is of poorer "quality" than that of the latter.   Since the control gluten was not affected adversely to the same extent as the purified gluten, this would suggest that the purified gluten was slightly modified.   Further studies on gluten from laboratory milled flours and on fat-free flours are needed.

Recommended Procedure for Preparing Purified Dried Gluten

1. *Gluten Preparation.*   Mix 1,000 g. flour and 800 ml. tap water at 30°C. for 20 min. in a suitable dough mixer.   Add 3,000 ml. tap water and stir for 2 min. with a high speed stirrer.   Separate the gluten on a screen and drain to approximately 500 g.   Divide into eight portions.

2. *Dispersion.*   Disperse each portion in 250 ml. of 0.005 N acetic acid for 5 min. with a Waring Blendor.   Stir in five drops n-octyl

alcohol at the end of mixing. Check the pH (5.0–5.2). (Total volume 2,500 ml., concentration about 8 mg. of nitrogen per ml. of dispersion.)

3. *Purification.* Centrifuge for 10 min. at 2,000 times gravity. (A continuous centrifuge can be used but conditions must be established that reduce the starch to the desired level.)

4. *Recovery.* Stir the combined dispersions and add 14 ml. saturated (filtered) calcium hydroxide per 100 ml. dispersion. Check the pH of the supernatant liquid and titrate to pH 6.5–6.8. Recover the gluten from the stirrer and by screening, if necessary. Further handling interferes with the following steps. Divide into four portions.

5. *Second Dispersion and Purification.* Disperse each portion in 250 ml. 0.01 *N* acetic acid for 5 min. with a Waring Blendor. Stir in 5 drops *n*-octyl alcohol at the end of mixing. (pH 5.5–5.7, total volume 1,500 ml., concentration about 13 mg. nitrogen per ml. dispersion.) Centrifuge and recover as before. N.B.—If freedom from starch is not important, the concentration can be increased to 20 mg. per ml. and this reduces the drying load.

6. *Vac-ice Drying.* Pour the dispersion into rust-resistant metal trays to a depth of 0.5 cm., freeze, and while it is still frozen evaporate at low pressure. Heat has to be applied to counteract cooling due to evaporation and the temperature should be maintained at −1°C. Drying requires 24–36 hr. if the pressure is kept below 1 mm. of mercury. The product can be rubbed through a 40-mesh sieve.

7. *Storing.* As a precaution, store the gluten in sealed containers in a refrigerator at about 5°C.

N.B.—Steps 1–5 inclusive do not require more than 3 hr.

Acknowledgments

Literature Cited

1. AITKEN, T. R., and GEDDES, W. F. The effect on flour strength of increasing the protein content by addition of dried gluten. Cereal Chem. **15**: 181–196 (1938).
2. CLENDENNING, K. A. Rapid determination of starch in crude gluten. Can. J. Research, C. **20**: 403–410 (1942).
3. COOK, W. H., and ROSE, R. C. Hydrolysis of gluten induced by the solvent. Can. J. Research **12**: 248–252 (1935).
4. HARRIS, R. H. A comparative study of some properties of dried glutens prepared from various types of wheat. Cereal Chem. **17**: 222–232 (1940).
5. HARRIS, R. H., and SIBBIT, L. D. The comparative baking qualities of hard red spring wheat starches and glutens as prepared by the gluten-starch blend baking method. Cereal Chem. **19**: 763–772 (1942).

6. Mangels, C. E., and Martin, J. J.   Peptization of wheat flour proteins by
    organic acids.   Cereal Chem. 12: 149–157 (1935).
7. McCalla, A. G., and Gralen, N.   Ultracentrifuge and diffusion studies on
    gluten.   Can. J. Research, C. 20: 130–159 (1942).
8. McCalla, A. G., and Rose, R. C.   Fractionation of gluten dispersed in sodium
    salicylate solution.   Can. J. Research 12: 346–356 (1935).
9. Olcott, H. S., and Mecham, D. K.   Protein-lipid complex formation during
    doughing of flours.   Lipoprotein nature of the glutenin fraction.   Cereal
    Chem. 24: 407–414 (1947).
10. Olcott, H. S., Sapirstein, L. A., and Blish, M. J.   Stability of wheat gluten
    dispersions toward reducing agents in the presence and absence of a gluten
    proteinase.   Cereal Chem. 20: 87–97 (1943).
11. Sandstedt, R. M., and Blish, M. J.   A new characterization of the gluten
    proteins.   Cereal Chem. 10: 359–366 (1933).
12. Shewfelt, A. L., and Adams, G. A.   Separation of starch and gluten.   III—A
    rapid method of separation from wheat flour.   Can. J. Research, F. 23:
    373–382 (1945).
13. Spencer, E. Y., and McCalla, A. G.   Fractional solubility of gluten in sodium
    salicylate solutions.   Can. J. Research, C. 16: 483–496 (1938).
14. Wood, T. B., and Hardy, W. B.   Electrolytes and colloids.   The physical state
    of gluten.   Proc. Roy. Soc. London, B. 81: 38–43 (1908).

NUMBERS, KINDS, AND SOURCE OF MOLDS IN FLOUR [1]

Clyde M. Christensen and Mortimer Cohen [2]

ABSTRACT

The mold counts of approximately five hundred samples of flour, col-
lected principally in commercial mills, ranged from several hundred to more
than 5,000 per g.   Three samples of washed wheat from one mill, collected
as the wheat went to the first break rolls, contained only a few hundred
molds per g., while the flours milled from these wheats contained up to
several thousand molds per g.   Flour collected from the interior of
spouts, roll housings and other mill machinery bore from several thousand
to several million molds per g., of the same species as were commonly
encountered in commercial flours.   The chief source of mold contamination
of flour appears to be molds growing and sporulating within the milling
system itself.   The predominant molds in most of the flours were *Asper-
gillus glaucus* and *A. candidus.*   Unidentified species of *Penicillium* made
up a major portion of the mold flora in only a few samples of commercial
flour.   Several other genera were found in most samples, but only in small
numbers.

The evidence now available, if not extensive, is at least sufficient to
indicate that freshly milled flour bears considerable numbers of differ-
ent bacteria, yeasts, and molds.   The literature dealing specifically

with molds in flour is scanty.   Barton-Wright (1) stated that freshly
milled flours contained on the average from 1,000 to 2,000 molds per
g.   The medium he used was not designed specifically to select
molds, and so the counts he reported probably were somewhat low.
Christensen (2) compared several technics and a number of different
media for the purpose of determining mold counts of flour.   He found
malt-salt agar to be preferable to the others tested, primarily because
it resulted in larger counts and a greater number of species, in the
majority of samples, than any of the other media tested.   James and
Smith (4) in a rather thorough study of the microflora of five Canadian
flours, obtained a higher mold count on malt-salt agar than on Czapek's
agar in all five samples.   They emphasized the need for adequate
replicates, as well as suitable sampling technics and selective media,
in determining the microfloral population of flour.   The chief aims of
the present work were to determine the number and kinds of molds
present in commercial wheat flours, and to explore some of the possible
sources of this contamination.   The data were obtained from assays
of approximately five hundred samples of flour, from 16 mills, over a
period of nearly four years, and thus is to be considered only as a
moderately extensive preliminary survey.

Materials and Methods

Collection of Samples.   Most of the samples of flour were collected
at the mills, although a few were obtained from bakeries.   At the
mills, the flour from different streams usually was obtained by inserting
a small, sterile paper bag into the spout to get the amount wanted.
With a few exceptions, each sample comprised from 4 to 16 ozs.

Determining Mold Population.   One gram of flour was weighed on
sterile filter paper on a torsion balance sensitive to 0.01 g., placed in a
4 oz. bottle containing 10 g. of sterile quartz sand and 100 ml. of sterile
water to which a wetting agent had been added in concentration of
approximately 1:10,000.   Previous tests had indicated that the
wetting agent in this concentration had no inhibitory effect on germina-
tion or growth of the molds concerned.   The mixture was then shaken
200 times, and 1 ml. portions immediately pipetted into each of two
to four sterile petri dishes.   Twenty ml. of malt-salt agar (20 g. malt
extract, 20 g. agar, 75 g. NaCl, 1 liter of water) cooled to 48–50°C.
were added, the dishes swirled to distribute the flour through the agar,
and allowed to stand until the agar hardened.   The dishes were then
stacked and covered to exclude air contaminants.   Occasionally flour
sterilized by heating to 180°C. for 4 hours was similarly sampled as a
control; these seldom averaged one mold colony per plate, indicating
that chance contamination was negligible.

The dishes were incubated in a laboratory at temperatures of 21–23°C. Colonies were counted after five to seven days. In most cases the dishes were examined both with the naked eye and microscopically, at magnifications of 10× to 30×.

Variation in Mold Counts of Flour from Different Mills. The mold counts of flours from 16 mills in 15 cities of 9 different states are presented in Table 1. A number of different types of flour were included, so that data probably indicate only the general range of mold contamination that is to be encountered. Some of these samples were

TABLE 1

Mold Counts of Wheat Flours from Different Mills

Mill No.	Location	Type of flour	Date collected		Molds per g.
1	Idaho	Cake	Aug. '46		200
2	Idaho	Bakers Patent	Aug. '46		500
3	Utah	Bakers Patent	Aug. '46		300
4	Colorado	Bakers Patent	Aug. '46		800
5	Colorado	Family Patent	Aug. '46		1,400
6	Kansas	Bakers Patent	Aug. '46		800
7	Kansas	Family Patent	Aug. '46		1,400
8	Kansas	Bakers Patent	Aug. '46		4,000
9	Missouri	Patent	Aug. '46,	sample 1	2,200
				sample 2	3,100
10	Missouri	Sponge	Aug. '46		2,900
11	Missouri	Bakers Patent	Aug. '46,	sample 1	2,600
				sample 2	3,300
				sample 3	4,800
				sample 4	5,200
12	Minnesota	Clear	Aug. '45		4,700
			Nov. '45		1,100
			Nov. '45		900
13	Minnesota	Family Patent	July '45		200
14	S. Dakota	Bakers Patent	Nov. '46		600
			Dec. '47		1,800
			Jan. '48		3,200
			Jan. '49		400
15	Oregon	Bakers Patent	Jan. '49		1,500
16	New York	Bakers Patent	Aug. '46,	sample 1	900
				sample 2	800

obtained from mills, some from bakeries. In all cases the moisture content of the flour was considered to be too low to permit significant increase in molds during the time that elapsed between samples and assaying them.

Variation in Mold Counts among Representative Streams. Fourteen streams in each of two mills and 21 in another were assayed, the first two in November, 1945, and the third in September, 1946. The data in Table 2 were selected from these samplings to illustrate the general range of mold contamination in the various streams of each of the three mills.

TABLE 2

MOLD COUNTS OF FLOUR FROM REPRESENTATIVE STREAMS IN THREE MILLS

Streams	Mill		
	1	2	3
1st break	66,000	2,500	2,800
2nd break	8,400		1,200
4th break	3,800	6,600	800
1st middlings			300
2nd middlings			500
6th middlings			200
1st scalp		3,900	
3rd scalp		6,400	
1st tailings	6,900		1,000
2nd tailings	7,400	9,200	1,700

Variation in Mold Counts among Different Streams of the Same Mills, and within the Same Stream at Different Times. In one mill, all of the streams that contributed to the "first clear" were sampled at intervals of four to eight hours through a period of 24 hours, during January, 1947, and the bran and shorts streams were sampled at the beginning and end of the 24 hour period. The aim was to determine whether some streams were contributing significantly more molds than others to the final product, and also to determine whether the portion of the seed going into flour had a higher or lower mold count than the portions not going into flour. Approximately 80 samples were assayed, and typical data are presented in Table 3. These were chosen to indicate the degree of mold contamination encountered, in this single test, in the various streams that contributed to the final flour and those that made up the portions not going into flour.

Variation within a Given Sample. To determine the variation within a given sample, five additional one-gram portions of the first middlings flour collected at 3 P.M. in January, 1947 (included in Table 3) were assayed. According to the first assay, this flour had a

TABLE 3

MOLD COUNTS OF THE SAME STREAMS IN ONE MILL AT INTERVALS OF 4 TO 24 HOURS, JANUARY 21–22, 1947

	1st Bk. flour	1st mid- dlings flour	1st tailings flour	1st clear flour	Bran	Shorts
Jan. 21, 11 A.M.	6,800	3,400	4,700	4,600	1,200	3,200
3 P.M.	4,300	800	6,400			
7 P.M.	5,700	1,700	6,300		2,600	
11 P.M.	8,900	2,400	4,600			
Jan. 22, 3 A.M.	12,800	5,000	8,300		3,000	
11 A.M.	8,000	1,400	6,200	3,300	700	⟩ 6,200

count of 800 molds per g.   Five plates were used for each of the five
replicates.   As determined by these replicates, the mold count of this
sample was, respectively, 800, 1,000, 1,050, 1,100, and 1,350 per g.,
with an average of 1,050.   Another sample which, according to the
original assay, had a mold count of 7,200 per g., was assayed by five
different workers.   Their results on this sample were 7,000, 6,800,
7,000, 7,300 and 7,200 per g.

Thus, variations due to technic and to unequal distribution of
spores within the sample probably may be expected to fall within a
range of 5% to 20%.   If dilutions are used that result in 20 to 50
colonies per plate, as was the case in most of the present work, the
variations between replicates of the same sample usually amounts to
no more than plus or minus 5% to 10%.

Source of the Molds Present in Flour.   Evidence presented elsewhere
(2) shows that wheats, as they come to the mill, may have a mold

TABLE 4

MOLD COUNTS OF 3 SAMPLES OF WHEAT BEFORE AND AFTER WASHING,
AND OF TYPICAL PRODUCTS MILLED FROM THEM

	Wheat sample number		
	1	2	3
Wheat before washing	2,800	3,100	1,900
Wheat after washing	300	400	500
Tempered wheat	300	400	500
Bran	2,600	1,900	1,200
Shorts	3,200	4,700	2,700
Patent	1,800	3,200	400

count of from 2,000 to 5,000 or more per g.   Data gathered in the
present tests support the contention that this is a "normal" mold
contamination of high grade wheats as they arrive at the mill.   To
determine whether these molds on the wheat seed were contributing
significantly to the mold contamination of flour milled from the same
seed, the mold counts of unwashed, washed, and tempered wheats, and
of the various fractions milled from these wheats, was determined.
The results are presented in Table 4.

While the data are limited in number, they are from samples
gathered at approximately annual intervals and thus represent different
varieties of wheat grown under widely different environmental condi-
tions in the Great Plains area.   Evidently, the mold count of high
quality wheat, after washing, usually amounts to only a few hundred
per g.   The greater mold counts of the bran and shorts as com-
pared with that of the washed wheat might be explained by the

hypothesis that most of the mold present in the seed is present as mycelium in the outer layers of the pericarp. In at least one of the samples, however, 100% of the mold count of the washed wheat was *Alternaria*, but *Alternaria* did not make up more than 5% of the count of the bran or shorts milled from this wheat. Also, the relatively high count of molds in two of the samples of patent flour obtained from wheat with a low mold flora as it went to the break rolls could not be explained by such a hypothesis. This suggested that molds common in flour were increasing and multiplying within the milling system itself.

To test this, various samples of flour were collected from the interior of roll housings, spouts, conveyers, and other places where the

TABLE 5

MOLD COUNTS OF FLOUR ADHERING TO INTERIOR OF MILL MACHINERY

Mill	Source of flour	Molds per g.
1	Conveyer of 8½ reel	88,000
2	Break roll housing	1,117,000
3	First break reel	16,000
	Inner side of cover of inspection opening in 2nd break shaker	1,500,000
4	1st break roll housing	4,700
	2nd break roll housing	11,000
	3rd break roll housing	726,000
	4th break roll housing	13,000
	5th break roll housing	2,456,000
	2nd midds. red. roll housing	364,000
	3rd midds. red. roll housing	892,000
	4th midds. red. roll housing	5,000
	5th midds. red. roll housing	1,014,000
	6th midds. red. roll housing	1,020,000
	Sizings reduction roll housing	3,360,000

relative humidity might be high enough to favor the increase of molds. The results are presented in Table 5.

The molds cultured in large numbers from this material were of the same species as those encountered as major contaminants in commercial flours from these and other mills. Most of the mold contamination of commercial flours that we have sampled appears to come from within the mill machinery itself. Additional proof of this is offered by a summary of the major types of mold contaminants of the flours we have assayed, as given below.

Principal Kinds of Molds Cultured from Commercial Flours. Approximately 20 species of molds, in eight genera, have been found in the flours so far sampled. Of these, *Aspergillus glaucus* and *A. candidus* have made up from 60% to 90% of the counts in the majority of samples, regardless of the location of the mill, the type of wheat milled,

TABLE 6

MOLDS ISOLATED FROM FLOUR

A. Prevalent in most samples
   Aspergillus glaucus
   A. candidus

B. Prevalent in occasional samples
   Penicillium sp.
   Aspergillus flavus

C. Present in small numbers in some samples
   Aspergillus ochraceus
   A. niger
   A. versicolor
   A. terreus
   Rhizopus nigricans
   Mucor racemosus
   Hormodendrum sp.
   Alternaria tenuis
   Helminthosporium sp.
   Fusarium sp.

or the time the samples were collected. In a few cases, species of *Penicillium* not yet identified have predominated. Other molds have been present only in small numbers, and then usually only in streams such as the purifier suction, where some contamination from outside air might be expected. The principal molds present in nearly all of the several hundreds of different samples we have assayed are those known to grow and sporulate at relative humidities between 75% and 85%. Thus they could be expected to prevail within most milling systems to a greater or less extent. The approximate composition of the fungus flora isolated by the writers from wheat flour is given in Table 6.

Longevity of Fungi in Flour. One sample of flour collected originally in a sterile tin container and one collected in a sterile paper bag were stored in the laboratory at room temperature for two to three

TABLE 7

MOLD COUNTS OF FLOUR AFTER STORAGE FOR 2 TO 3 YEARS

Sample	Date tested	Molds per g.	Composition of fungus flora
1. Bakers Patent	Feb. '46	3,600	*Aspergillus glaucus* 75% *A. candidus* 15% *Penicillium* 10%
	Sept. '47	2,700	Same as in 1946
	Jan. '49	1,400	Same as in 1946
2. 4th Break	Feb. '47	7,200	Principally *Aspergillus candidus*
	Jan. '49	7,200	Same as in 1947

years, and periodically assayed for molds. The original and subsequent mold populations of these are given in Table 7.

Obviously the mold spores present in these flour samples lost their viability rather slowly. This perhaps is to be expected, since the spores of some of these molds are known to remain viable, in dry materials, for some years.

Discussion

The data gathered in this study indicate that commercial flours generally are contaminated with from several hundred to several thousand viable mold spores per g., or from 100,000 to several million per pound. The flour from some mills appears to have a consistently higher mold count than that from other mills. The mold count of flour from a given mill seems to be determined more by conditions that prevail within the milling machinery than by the wheat from which the flour is milled. There is, at present, no evidence to indicate whether any of the molds found in the present study, in the numbers they were found, have any significant effect on flour quality. In some of the flours, considerable numbers of unidentified yeasts were present. The work of James and Smith (3) indicates that flours may contain various kinds of bacteria and yeasts as well as molds. Eventually it would seem desirable to determine whether the microfloral population of flour—bacteria, yeasts, and fungi—might, at times, affect the quality of flour for baking purposes.

Literature Cited

1. BARTON-WRIGHT, E. C. Studies on the storage of wheaten flour. III. Changes in the flora and the fats and the influences of these changes on gluten character. Cereal Chem. 15: 521–541 (1938).
2. CHRISTENSEN, C. M. The quantitative determination of molds in flour. Cereal Chem. 23: 322–329 (1946).
3. CHRISTENSEN, C. M., and GORDON, DOROTHY R. The mold flora of wheat and corn and its relation to heating of moist grain. Cereal Chem. 25: 40–51 (1948).
4. JAMES, NORMAN and SMITH, K. N. Studies on the microflora of flour. Can. Jour. Res. 26: –C– 479–485 (1948).

COMMUNICATION TO THE EDITOR

Preparation of Spray Dried Wheat Gluten [1]

DEAR SIR:

Drying of wheat gluten to preserve its bread baking qualities may be accomplished by exposure of the gluten in films or small pieces to air at room temperature, by evaporation of water from the frozen material at low pressures (lyophilization), or by vacuum oven drying at temperatures of 30–40°C. Of these methods, only the last is applicable to commercial operations at present and low capacity and long drying periods make it of limited value.

The finding that wheat gluten can be readily dispersed in 0.01 N acetic acid to give solutions containing up to 15% dry matter[2] suggested the possibility of spray drying these solutions to recover the gluten.

Gluten was dispersed in 0.01 N acetic acid, centrifuged to remove starch, precipitated by neutralizing the acetic acid and redispersed in 0.01 N acetic acid to give a concentration of approximately 12%.[2] The pH of the dispersions varied between 5.6–5.8 depending on the amount of gluten dispersed. The gluten dispersions were delivered to a laboratory spray drier of the conventional cyclone type described in detail elsewhere.[3] The spray in this small drier was produced by pumping the dispersion with a small centrifugal pump to the spray nozzle where it was atomized by an air jet operating at 20 p.s.i. The hot air used for drying was supplied at the rate of 90 cu. ft. per min. Inlet temperatures were varied from 250°F. (121°C.) to 300°F. (149°C.) (higher temperatures could not be obtained with the heating equipment available), and exhaust temperatures were varied from 175°F. (79°C.) to 225°F. (107°C.) (lower exhaust temperatures failed to dry material adequately). Within the range of air inlet and exhaust temperatures found practicable the gluten was recovered as a fine powder of about 2.5% moisture content. Most of the gluten was collected from the main drying cone but, owing to its low density, a considerable proportion was carried over and recovered from the collecting system.

[1] N.R.C. No. 2089.
[2] Lusena, C. V. Preparation of dried native wheat gluten. Cereal Chem. 27: 167–178. 1950.
[3] Woodcock, A. H. and Tessier, H. A laboratory spray drier. Can. J. Research, F. 21: 75–77. 1943.

On addition of water the dried gluten immediately formed a sticky cohesive mass similar in physical characteristics to the original gluten. Retention of native properties in the dried gluten was assessed by solubility in dilute acetic acid solution and by baking tests. All gluten samples had 95% solubility in 0.005 N acetic acid indicating that little denaturation had taken place. The baking test consisted of addition of the gluten to a soft white spring wheat (containing 8.3% protein, 14% moisture basis) to raise the protein content to 13.5% and baking by the malt phosphate formula.[4]

TABLE I

BAKING QUALITY OF WHEAT GLUTEN PREPARED BY SPRAY DRYING
UNDER VARIOUS CONDITIONS

(Gluten dispersions used contained approximately 12% dry matter)

Sample no.	Operating conditions			Baking quality		
	Drying temp., °F.		Dried product, g./hr.	Loaf volume, c.c.	Crumb*	
	Inlet	Exhaust			Color	Texture
Control (air-dried gluten)	—	—	—	680	4.5	4.5
Vac-ice dried gluten	—	—	—	640	5.0	5.5
Spray dried glutens						
1	300 (149°C.)	175 (79°C.)	396	680	5.5	5.5
2	300 (149°C.)	200 (93°C.)	274	640	5.0	6.0
3	300 (149°C.)	225 (107°C.)	209	575	4.5	5.0
4	275 (135°C.)	175 (79°C.)	274	640	5.5	6.0
5	275 (135°C.)	200 (93°C.)	187	615	5.0	5.5
6	250 (121°C.)	200 (93°C.)	122	600	5.0	5.0

* The Grain Research Laboratory, Winnipeg, uses the following verbal descriptions for both color and texture: excellent, 8.6–10.0; very good, 7.0–8.5; good, 5.5–6.9; fair, 4.5–5.4; poor, below 4.5.

Conditions for the drying operation and results of the baking tests are given in Table I. They show that loaf volume decreased with increasing exhaust temperatures at both the 300°F. (149°C) and 275°F. (135°C.) inlet temperatures. None equalled the control in loaf volume, but this is attributed to the use of 0.01 N acetic acid for dispersing the original gluten as it was later shown that 0.005 N acid gave large loaf volumes,[2] equivalent to those obtained with gluten preparations dried from the frozen state. An inlet air temperature of 300°F. (149°C.) and exhaust temperature of 175°F. (79°C.) gave the highest drying capacity and a slightly superior product.

[4] Aitken, T. R., and Geddes, W. F. The effect on flour strength of increasing the protein content by the addition of dried gluten. Cereal Chem. 15: 181–196 (1938).

While starch-free gluten was used in the present study, a crude gluten containing 10–15% starch could be used.   These results show the feasibility of spray drying solutions of wheat gluten to yield a dry product that retains its bread baking properties.

November 8, 1949

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EDITORIAL POLICY

SUGGESTIONS TO AUTHORS

General. From January 1, 1948, an abstract will be printed at the beginning of each paper instead of a summary at the end, references will be numbered to provide the option of citing by number only, and date of receipt, author's connections, etc., will be shown in footnotes. Except on these points, authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6: 1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* 40: I–CCIX. 1946.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a group of related studies, each made with different materials or methods, may require a separate section for each study, with subsections for materials and methods, and for results, under each section. Center headings are used for main sections and italicized run-in headings for subsections, and headings should be restricted to these two types only.

Tables. Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side headings should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often useful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

Figures. If possible, all line drawings should be made by a competent draftsman. Traditional layouts should be followed: the horizontal axis should be used for the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be $\frac{1}{16}$ to $\frac{1}{8}$th inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

All original figures should be submitted with one set of photographic reproductions for reviewers, and each item should be identified by lightly writing number, author, and title on the back. Cut-lines (legends) should be typed on a separate sheet at the end of the manuscript. "Preparation of Illustrations and Tables" (*Trans. Am. Assoc. Cereal Chem.* 3: 69–104. 1945) amplifies these notes.

Text. Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

Editorial Style A.A.C.C. publications are edited in accordance with *A Manual of Style,* University of Chicago Press, and *Webster's Dictionary.* A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

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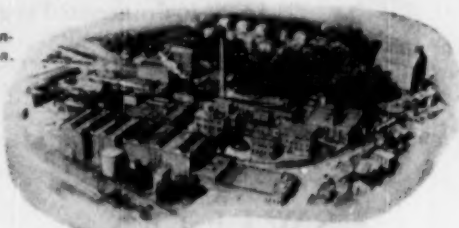
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